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HYBRIDIZATION BETWEEN *CYPRINODON ATRORUS* AND *C. BIFASCIATUS*: HISTORY, PATTERNS, AND DYNAMICS

by

Evan Wiley Carson

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

ARIZONA STATE UNIVERSITY

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HISTORY, PATTERNS, AND DYNAMICS

by

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has been approved

January 2005

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ABSTRACT

This study sought to address whether environmental variation is important to the dynamics of hybridization between two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, endemic to the Cuatro Cienegas basin, Coahuila, Mexico. To address this question, the history of hybridization between *C. atrorus* and *C. bifasciatus* was first investigated by conducting a basin level survey of nuclear gene (CK-A7, RAG-1, and TPI-B4) and mitochondrial (cyt*b*) variation in these two species. This analysis revealed that at the nuclear gene level, *C. atrorus* and *C. bifasciatus* are diagnostically different. Nuclear gene variation within these species suggests that the largest and oldest populations occur in the central and western basin for *C. atrorus* and *C. bifasciatus*, respectively. At the mitochondrial level, there has been complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus*. Subsequent to this replacement there has been diversification of mitochondrial haplotypes along hydrogeographic regions, rather than by species. For the second facet of this project, reciprocal transplant experiments were conducted with *C. atrorus* and *C. bifasciatus* to test whether these species differ significantly in their environmental tolerances. Results from this study confirm the presumed euryplasticity of *C. atrorus* and stenoplasticity of *C. bifasciatus*, and suggest that introgression of *C. bifasciatus* genes into *C. atrorus* populations is limited at least in part by environmental tolerance differences between these species. For the final aspect of this project, spatio-temporal analyses of the relationship between environmental and genetic variation in a hybrid zone between *C. atrorus* and *C. bifasciatus* were conducted. On a spatial scale, there was a positive correlation between *C. atrorus*-specific allele frequency and highly variable environments, while there was a

positive correlation between increased *C. bifasciatus*-specific allele frequency and thermally buffered environments, a pattern at least partially explained by the physiological characteristics of these species. On a temporal scale, allele frequency in the hybrid zone tracked environmental change over time, with *C. bifasciatus*-specific allele frequency declining in both cold and hot conditions, but increasing when thermal regimes of habitats acquired a spring-like thermal profile. Conversely, *C. atrorus*-specific allele frequency increased as physicochemical environments became more variable.

For

My Parents

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PROLOGUE

Considerable controversy has surrounded the role of hybridization in evolution, particularly in animal systems. Recent analyses have built a growing consensus that hybridization can play an important role in animal as well as plant systems. However, critical gaps remain in understanding the spatio-temporal relationships between environmental and genetic variation in many plant and animal systems. This study focused on natural hybridization between the pupfish species *Cyprinodon atrorus* and *C. bifasciatus*, and was designed to contribute to our understanding of the evolutionary consequences of hybridization in animals. In particular, hybridization between *C. atrorus* and *C. bifasciatus* presents an excellent natural laboratory for investigating the role of exogenous (environmental) selection in the structure and maintenance of hybrid zones, a focus that has received only minor attention in animal relative to plant systems. This investigation primarily explored the history, patterns, and dynamics of hybridization between *C. atrorus* and *C. bifasciatus*, with particular emphasis on spatio-temporal relationships between environmental and genetic variation in hybrid zones between *C. atrorus* and *C. bifasciatus*. This study is broken into five chapters: background and introduction to the system (Chapter 1), mitochondrial and nuclear population genetics of *Cyprinodon atrorus* and *C. bifasciatus* (Chapter 2), reciprocal transplant experiments with *C. atrorus* and *C. bifasciatus* (Chapter 3), spatio-temporal relationships between nuclear gene and environmental variation in a hybrid zone between *C. atrorus* and *C. bifasciatus* (Chapter 4), and synthesis (Chapter 5).

Chapter 1 provided the basic background regarding the role of endogenous and exogenous selection in plant and animal hybrid zones, detailing the arguments for and

against the roles of these types of selection in hybridization. Particular attention was paid to the need for spatio-temporal analyses of hybridization and the role of hybridization in the evolutionary dynamics of animals. This chapter also identified the need for study of environmental influences on the regulation of animal hybrid zones. In addition, this chapter introduced the system of hybridization between *Cyprinodon atrorus* and *C. bifasciatus* and laid the groundwork for using this system to study hybridization in general by presenting arguments for why this system of hybridization is appropriate for addressing issues that extend beyond this system per se.

In chapter 2, I constructed an evolutionary framework for understanding how hydrogeography and hybridization have influenced mitochondrial and nuclear population genetic patterns in these species. This chapter provided historical information for determining past distributions of these species in the basin, a rigorous basis for understanding how hybridization between *C. atrorus* and *C. bifasciatus* has influenced present day mitochondrial and nuclear population genetic patterns. I also proposed an evolutionary scenario in which hydrogeography and hybridization have interact to regulate patterns of mitochondrial variation and distributions in this system.

Because the primary goal of this project was to identify potential environmental selection agents important in structuring hybridization between these species, Chapter 3 addressed how one exogenous factor, differences in environmental tolerance, may be important in shaping hybridization between *C. atrorus* and *C. bifasciatus*. In this chapter, results from summer and a winter reciprocal transplant experiments with *C. atrorus* and *C. bifasciatus* were related to the potential importance of environmental selection in hybrid zones between these species. The hypothesis was that the presumably stenoplastic

C. bifasciatus would demonstrate significantly lower tolerance of environmental variation and severity than would euryplastic *C. atrorus*. If differences in environmental tolerance are important, then *C. bifasciatus* should have exhibited poor survival in the severe habitats of *C. atrorus*. Alternatively, *C. atrorus* should have had high survival in its own habitat as well as that of *C. bifasciatus*. Information gathered from this experiment allowed for better interpretation of hybrid response to environmental change (Chapter 4).

Because hybrid zones are dynamic rather than static systems, Chapter 4 was used to investigate the relationships between seasonal/inter-annual environmental change and nuclear gene variation in a hybrid zone between *C. atrorus* and *C. bifasciatus*. In this study, specimens were collected throughout the hybrid zones in summer, winter, and spring for three consecutive years in order to determine how genetic variation responded to seasonal environmental change. This chapter relied heavily on findings from reciprocal transplant experiments (Chapter 2) and presented four hypotheses relative to how hybrids should shift distribution with particular seasonal changes in environmental condition. We demonstrated that because hybrids predictably track environmental change, exogenous (environmental) selection must play a critical role in governing the dynamics of hybridization between *C. atrorus* and *C. bifasciatus*.

Chapter 5 provided a synthesis of information and findings from Chapters 1-4 to provide a better overall understanding of the history, patterns, and dynamics of hybridization between *C. atrorus* and *C. bifasciatus*. The synthesis also laid out future research avenues for studying hybridization between *Cyprinodon atrorus* and *C. bifasciatus*, which included proscriptions for more detailed study of the ecology and ecological/evolutionary genetics of these species. Finally, the synthesis also tied together

ways that this study is relevant to the overall importance of hybridization in the evolution of animal systems.

CHAPTER 1

Introduction

A central challenge for evolutionary biologists is to synthesize observed evolutionary processes and patterns into general explanations of organismal diversity. Hybrid zones provide unique opportunities to address many questions relevant to this challenge and therefore have been subject to intense investigation in plant and animal systems (Anderson 1948, 1949, Anderson and Stebbins 1954, Barton and Hewitt 1985, Hewitt 1988, Harrison 1990, Arnold 1992, Harrison 1993, Howard 1993, Arnold 1997). Nonetheless, opinions concerning the evolutionary importance of hybridization have differed substantially between botanists and zoologists. While botanists have long championed a creative role for hybridization in plants (Lotsy 1916, Anderson 1948, 1949, Anderson and Stebbins 1954, Arnold 1992, Rieseberg 1993; but see Heiser 1949), zoologists have traditionally considered hybrids maladapted, and therefore of little relevance to animal evolution (Fisher 1930, Mayr 1963, Barton 1979, 1980, 1983, Barton and Hewitt 1985, 1989, Hewitt 1988; but see Dobzhansky 1941). Results from recent analyses of hybridization demand reappraisal of its significance in animal systems (DeMarais et al. 1992, Dowling and DeMarais 1993, Arnold 1997, Dowling and Secor 1997) and call for greater emphasis on how environmental factors contribute to the dynamics and fate of introgressed populations.

Potential evolutionary consequences of hybridization are numerous and include transfer of adaptive variation between species (Arnold 1997), production of novel adaptations (Anderson 1949, Stebbins 1959, Lewontin and Birch 1966, Grant 1981), and creation of new species through recombination or reinforcement (Dobzhansky 1941,

Howard 1993, Rieseberg et al. 1995, Arnold 1997, Dowling and Secor 1997). Thus, hybrid zones can act as conduits for evolutionary change within parental populations or foster evolutionary diversification from within intervening recombinant populations. Implicit in these scenarios is that evolutionary change is effected at least in part by exogenous (environmental) selection. Several authors have addressed the importance of environmental influences on hybrid zone maintenance. In a cricket hybrid zone Harrison (1986) observed that genetic structure was correlated with mosaic partitioning of parental habitats (sand vs. loam soil types). With this evidence he argued that such "mosaic" hybrid zones are maintained by environmental selection against hybrids, not inherent genetic inferiority of such individuals. An extension of this concept is provided by Moore (1977) in his Bounded Hybrid Superiority model of hybrid zone structure. In this model, narrow hybrid zones result from equivalent or higher fitness hybrids in ecotones because they possess the necessary intermediate characteristics to exploit such niches. Howard (1993) further suggested that admixed subpopulations within the interior of these zones are subject to little contact with "pure" parental genotypes, and could thus become locally adapted. As a consequence, mosaic hybrid zones may serve as centers for evolutionary diversification (Jiggins and Mallet 2000).

Several recent analyses of plant and animal hybrid zones not only identify environmental influence as critical to hybrid zone dynamics, but provide cogent evidence that recombinant genotypes can harbor evolutionary potential. Such effects have been demonstrated by Arnold and his colleagues in a series of experiments on *Iris* hybrid zones. In a detailed analysis of genotype-environment associations in a hybrid zone

involving *Iris brevicaulis* and *I. fulva*, Cruzan and Arnold (1993) concluded that environmental influence was an important factor in the maintenance and structure of this hybrid zone. In other experiments, Cruzan and Arnold (1994) and Emms and Arnold (1997; see also Arnold and Hodges 1995) demonstrated that certain recombinant classes of *Iris* were more fit than others, and that not all recombinant genotypes were unfit relative to parental taxa. In fact, some hybrid genotypes were more fit than parental ones even in parental habitats. This effect also has been demonstrated for animals. In Galapagos finches (*Geospiza*), Grant and Grant (1992, 1993, 1996) demonstrated a temporally dependent selective advantage for hybrids under certain environmental conditions associated with El Nino/La Nina climatic cycles.

Certain recombinant genotypes can also exploit habitats that are novel with respect to parental species or other classes of hybrids (Arnold 1992, 1997, Cruzan and Arnold 1993, 1994, Arnold and Hodges 1995). In an *Iris* hybrid zone (*I. brevicaulis* x *I. fulva*), Cruzan and Arnold (1993) found *I. fulva*-like recombinants occupied habitats that did not contain *I. fulva*, *I. brevicaulis*, or *I. brevicaulis*-like genotypes. They concluded that the particular recombinant constitution of *I. fulva*-like hybrids was a causal factor in exploitation of the new habitats. In animals, Lewontin and Birch (1966) presented evidence that introgressive hybridization between the tropical fly *Dacus tryoni* and a fly adapted to extreme environments, *D. neohumeralis*, could have enabled recombinant populations of *Dacus tryoni* to expand into novel, environmentally extreme habitats. Consistent with patterns of introgressed populations occupying novel habitats is the striking similarity observed for habitat preferences of known hybrid taxa. At least four

hybrid derived plant taxa, *Helianthus anomalus*, *H. deserticula*, *H. paradoxus*, and *Iris nelsonii*, occupy habitats unlike those of their progenitors (Arnold 1993, Arnold et al. 1990, 1991, Rieseberg 1993, 1997, Rieseberg et al.1990, 1995).

Despite compelling evidence for a significant environmental component to structure, maintenance, and fate of many introgressed populations, most investigations of environmental influence on hybrid zone dynamics are limited to static, spatial characterization of hybrid fitness (Cruzan and Arnold 1994, Emms and Arnold 1997) or correlation of morphology/genotype to habitat (Rand and Harrison 1989, Cruzan and Arnold 1993, Sites et al. 1995). Temporal tracking of hybrid zone movement and dynamics has been investigated by several authors (Moore and Buchanan 1985, Arntzen and Wallis 1991, Howard et al 1993, Moore and Price 1993, Scribner 1993, Scribner and Avise 1994a, 1994b), but these investigations did not consider seasonal and annual environmental variation. The need for analyses of hybrid response to temporal environmental variation is clearly demonstrated in studies of Galapagos finches by Grant and Grant (1992, 1993, 1996), where dramatic shifts in hybrid fitness and abundance occurred in association with cyclical environmental changes caused by the El Nino/La Nina oscillation. Under certain environmental conditions, hybrid finches were shown to possess higher fitness than parental species, information that would likely have been missed without study over temporal scales.

Hybridization between two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, presents an excellent opportunity to investigate the influence of seasonal and annual environmental fluctuation on the evolutionary potential of recombinant

populations. These species are endemic to the ecologically complex Cuatro Cienegas basin, Coahuila, Mexico (Figure 1; Miller 1968, Minckley 1969). In this system, diverse aquatic habitats are connected through physicochemically constant thermal springs that flow into marshes, lagunas and ephemeral playas, which demonstrate moderate to severe physicochemical variability over spatial, seasonal, and annual scales (Minckley and Cole 1968). *Cyprinodon atrorus* is a member of the informally recognized *C. eximius* species complex (Miller 1968, Echelle and Echelle 1998, Echelle et al. 2005) and is typical of most *Cyprinodon* in its ecology (Arnold 1972), behavior (Itzkowitz 1967, Minckley and Itzkowitz 1967, Itzkowitz and Minckley 1969, Minckley and Arnold 1969), and broad physiological tolerances (Miller 1968, Minckley 1969, and Arnold 1972). *Cyprinodon atrorus* primarily inhabits marshes, seasonal pools, and permanent evaporative lagunas, all of which are subject to extreme physicochemical severity and variability. *Cyprinodon bifasciatus* is a basal member of the genus (Miller 1968, Echelle and Echelle 1998) and is unusual among pupfishes in its behavior (Arnold 1972), ecology (Miller, 1968, Minckley 1969, Arnold 1972), and presumably narrow physiological tolerances (Arnold 1972). In contrast, this species is restricted in winter to thermal springs and thermally buffered reaches of spring outflow (Minckley 1969, Arnold 1972).

In spring and summer, when downstream segments of outflow approach the physicochemical properties of their thermal sources, *C. bifasciatus* expands its range downstream and into contact with *C. atrorus* (Minckley 1969). Under natural conditions, hybrid zones between *C. atrorus* and *C. bifasciatus* occur in intermediate reaches between thermal springs and terminal lagunas or marshes. Habitat structure within these

hybrid zones is generally a mosaic of more physicochemically intermediate pool, marsh, laguna, and stream habitats in which *C. atrorus* and *C. bifasciatus* largely segregate by habitat (Minckley 1969, Arnold 1972, Ludlow 2000, Carson pers. obs.). Hybridization between these species (including mating contributions by hybrid offspring) occurs in shallow pool and marsh habitats that meet the preferred breeding territory requirements of males of both species and hybrids (Ludlow 2000, Carson pers. obs.). Local hybrid swarms predominate in some habitats, but hybrid composition varies considerably among local populations in hybrid zones (Carson per. obs.). General distributions of hybrids are consistent with experimental observations that crosses between *Cyprinodon* adapted to constant, thermal springs and those adapted to physicochemically variable habitats produce hybrids with intermediate physiological tolerances (Hirshfield et al. 1980).

Summary

Natural hybridization between *Cyprinodon atrorus* and *C. bifasciatus* presents an excellent system for investigation of the importance of environmental (exogenous) selection on the structure and dynamics of animal hybrid zones. As such, knowledge obtained from this system will aid efforts to gain a general understanding of factors most critical to the structure and dynamics of hybridization in animal systems. The strengths of the system of hybridization between *C. atrorus* and *C. bifasciatus* lie in both tractability (small basin) and the ease of experimental manipulation. Consequently, this provides an excellent experimental platform for testing critical hypotheses of hybridization in animals. Findings from this study will not only provide a better

understanding of hybridization between these species, but of hybridization in animals in general. This system also offers a host of opportunities for future research into the hybridization, ecology, and evolution of *C. atrorus* and *C. bifasciatus*.

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CHAPTER 2

Influence of hydrogeographic history and hybridization on the distribution of genetic variation in the pupfishes *Cyprinodon atrorus* and *C. bifasciatus*

Abstract

The evolutionary importance of hybridization in animals has been subject to much debate. Recent analyses provide strong evidence that environmental factors can play a critical role in animal hybrid zones, thus opening the possibility that hybridization is of evolutionary importance in animal systems. This study sought to disentangle the influences of hydrogeographic history and hybridization on the present distribution of nuclear and mitochondrial DNA variation in two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*. Results from this study indicate that there has been limited introgression of nuclear genes; however, mtDNA introgression has been substantial, with complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus*. Subsequent to this replacement, there has been diversification of mitochondrial haplotypes along major geographic regions in the basin. Evidence was also found that mitochondrial replacement follows a predictable, cyclical pattern in this system, with isolation and diversification followed by re-contact and replacement of *C. bifasciatus* mitochondrial haplotypes by those of *C. atrorus*. This pattern is best e xplained by a combination of a numeric bias towards *C. atrorus* and mating site selection rather than selection *for C. atrorus* mitochondrial genome. These results demonstrate the important role hybridization can play in evolution.

Introduction

A central challenge for evolutionary biologists is to synthesize observed evolutionary processes and patterns into general explanations of organismal diversity. Hybrid zones provide unique opportunities to address many questions relevant to this challenge and therefore have been subject to intense investigation in plant and animal systems (Anderson 1948, 1949, Anderson and Stebbins 1954, Barton and Hewitt 1985, Hewitt 1988, Harrison 1990, 1993, Arnold 1992, 1997, Howard 1993). Nonetheless, opinions concerning the evolutionary importance of hybridization have differed substantially between botanists and zoologists. While botanists have long championed a creative role for hybridization in plants (Lotsy 1916, Anderson 1948, 1949, Anderson and Stebbins 1954, Arnold 1992, Reiseberg 1993; but see Heiser 1949), zoologists have traditionally considered hybrids maladapted, and therefore of little relevance to animal evolution (Fisher 1930, Mayr 1963, Barton 1979, 1980, 1983, Barton and Hewitt 1985, 1989, Hewitt 1988). However, results from recent analyses of hybridization indicate that reappraisal of its significance in animal systems is critical (DeMarais et al. 1992, Dowling and DeMarais 1993, Arnold 1997, Dowling and Secor 1997).

Potential evolutionary consequences of hybridization are numerous and include transfer of adaptive variation between species, production of novel adaptations (Anderson 1949, Stebbins 1959, Lewontin and Birch 1966, Grant 1981), and creation of new species through recombination or reinforcement (Howard 1993, Arnold 1997, Dowling and Secor

1997). Hybridization can also obscure relationships between species through introgression, making it difficult to accurately interpret population history. For studies that aim to interpret broad-scale biogeographic and population genetic patterns within species, it is important that elements likely to have significant, distorting influences on population genetic structure be identified. Introgressive hybridization commonly disrupts or obscures population genetic structure by inflating genetic variance within species and reducing perceived divergence between species. Estimation of the impact of introgressive hybridization is particularly critical in many cases involving endangered species because effective management may depend on accurate assessment of historical influences on present population genetic patterns. In this study, we consider how present population genetic variation in two endangered pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, endemic to Cuatro Cienegas, Coahuila, Mexico, have been shaped by hydrogeography and introgressive hybridization.

Hydrogeography

Cuatro Cienegas is a small (~840 km^2) intermontane basin in the Chihuahuan desert, Coahuila, Mexico and was declared a Natural Protected Area by the Mexican government in 1994 due to its exceptional biodiversity and high levels of endemism. The basin is divided into eastern and western lobes that are partially separated by the plunging anticline of the Sierra de los Pinos (de San Marcos) (Figure 1). Associated with the bajada of the northern tip of this mountain is a series of thermal springs that arise from fissures along an active fault (Minckley 1969). Springs are represented by either isolated

limnocrenes or outflow into riverine systems that terminate in large evaporative lagunas or cienegas on the basin floor (barrial). Along the transition from spring to terminal habitats there is typically a steep environmental gradient, ranging from near physicochemical constancy (pH, salinity, temperature) of thermal springs and upper, environmentally buffered riverine habitats to severe daily and/or seasonal physicochemical fluctuations within peripheral environments and terminal evaporative lagunas/marshes. Middle and lower riverine reaches, as well as interfaces with peripheral habitats, are environmentally intermediate.

Present day hydrogeographic relationships among major systems are poorly understood due to limited mapping of epigean connectivity and uncharted, but presumably extensive subterranean networks thought to characterize the karstic sub-surface. A preliminary hypothesis of surficial drainage patterns (Minckley 1969) divides the basin into seven major drainage systems (Figure 1): 1) Rio Churince, 2) La Becerra/Rio Garabatal/Juan Santos, 3) Rio Mesquites, 4) Rio Puente Chiquita, 5) Escobeda/Tio Candido/Los Hundidos, 6) Santa Tecla, and 7) Rio Salado de los Nadadores. The Rio Churince and Becerra systems are separated by dunes and a low travertine sill (Minckley 1969), and thus not presently connected at the surface. Minckley noted that La Becerra and Rio Mesquites systems are likely connected through subterranean channels, but no surface connection currently exists. The Rio Mesquites and Rio Puente Chiquita are thought to have had surface connections in periods of high water, as both once emptied into the same terminal laguna (Las Playitas). Similarly, Escobeda/Tio Candido/Los Hundidos and Santa Tecla drainages are thought to have

emptied into the same barrial system (Los Gatos) (Minckley 1969). Based on travertine deposits, the spring at Escobeda may also have flowed into the Rio Mesquites, thus forming a pivotal position between the Rio Mesquites system and systems to the southeast (i.e., Tio Candido and Los Hundidos). The Rio Salado de los Nadadores is the easternmost drainage in the basin and is now dry; therefore, it will not be further discussed.

Although the exact nature and extent of historical connections between these hypothesized drainage systems are not known, it is thought that there was considerable connectivity over much of the basin during glacial episodes of the Plio-Pleistocene (Minckley 1969). This is supported by a recent test of the seven drainage systems hypothesis by Moline et al. (2004), who found no association between allozyme variation and drainage in the endemic snail *Nymphophilus minckleyi*. While they suggested that alternate drainage patterns may exist they provided no alternate hypotheses. However, *N. minckleyi* has a widespread distribution and occupies a diverse array of habitats, making it less likely than other endemic snails (e.g., the thermal spring endemic *Mexithauma quadripalludium* or intermediately distributed *Mexipyrgus churinceanus*) or headspring species of fishes (e.g., *Cyprinodon bifasciatus*) to show population genetic structure across drainages. In fact, pollen records indicate that the basin has maintained an ecologically equivalent flora for the last 30,000 to 40,000 years (Meyer 1973), suggesting that lacustrine habitats in the Late Pleistocene may have been limited. In any case, there is evidence that the basin has experienced a natural decline in water level during the Holocene, and numerous extinct surface connections are easy to identify in satellite

photographs of the basin (Minckley 1969). This evidence, in combination with known present day (or historically recorded) surface connections, suggests that at the very least the southeastern portion of the basin has been isolated from the remainder of the valley for a considerable period. In contrast, the central and western portions of the basin have probably had more recent connectivity over the surface.

Cyprinodon atrorus and C. bifasciatus

The two endangered pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, are locally abundant, but considered endangered due to their limited geographic ranges. Hybridization between these species represents one of the few modern cases of natural hybridization within *Cyprinodon*, although hybridization is thought to have been historically episodic and evolutionarily important within the genus (Echelle et al. 2005). Based on morphological differences, Miller (1968) considered *C. atrorus* and *C. bifasciatus* to be distantly related, with *C. atrorus* representing a more recently derived species and *C. bifasciatus* an early offshoot of *Cyprinodon* evolution. Phylogenies based on allozymic characters have corroborated the distant relationship between these species (Echelle and Echelle 1998); however, a comprehensive survey of mitochondrial and nuclear gene sequence variation has not been conducted. Such a survey could prove valuable in addressing phylogeographic history of *C. atrorus* and *C. bifasciatus* in the basin, provide insights into the consequences of hybridization between these species, and reveal valuable information for understanding historical connections among different hydrogeographic regions in Cuatro Cienegas. In turn, this information will add to the

general understanding of hybridization in animals and aid in management of these species.

Cyprinodon atrorus and *C. bifasciatus* occupy opposite extremes along steep environmental gradients (pH, salinity, temperature). *Cyprinodon atrorus* is typically found in the most severe and fluctuating environments associated with the basin floor (barrial) and its distribution generally surrounds that of *C. bifasciatus*, which is limited to physicochemically benign thermal springs and environmentally buffered riverine habitats. These species are vastly different in their faunal associations (Miller 1968, Minckley 1969), environmental tolerances (Chapter 3), and behavioral ecology (Arnold 1972). Though *C. atrorus* and *C. bifasciatus* generally are found at opposite ends of this environmental continuum, their ranges overlap in environmentally intermediate habitats. In these regions, hybridization is extensive (predominated by advanced backcrosses; Chapter 4).

Present day hybridization between *Cyprinodon atrorus* and *C. bifasciatus* occurs in two major zones: Rio Churince where habitats are essentially unaltered by human activity, and cienegas at the origin of Canal Tio Julio, where habitats have been extensively modified and hybridization has been expanded due to this human activity (Carson pers. obs.). In addition to these major systems of hybridization, innumerable opportunistic matings occur between *C. atrorus* and *C. bifasciatus* along the interfaces of stable/severe habitats associated with the Rio Mesquites (Carson, pers. obs.). Historically, however, hybridization may have been much more widespread, as connectivity between these species was probably greater than at present, especially

during the more mesic periods that pre-dated the Holocene. Natural aridification of the basin has since limited contact between these species and has also obscured historical connections within and between these species. To assess the role of hydrogeographic history and hybridization in the evolution of *Cyprinodon atrorus* and *C. bifasciatus*, variation in mitochondrial (cytochrome *b* [cyt*b*]) and nuclear (creatine kinase intron A7 [CK-A7], recombination activation gene intron 1 [RAG-1], and triosephosphate isomerase intron B4 [TPI-B4]) sequences were characterized.

The expected outcomes need to be evaluated at two levels. If introgressive hybridization has had an important influence on these taxa, species boundaries would be obscured and underlying patterns of genetic variation would only reflect the hydrogeographic history of the region. If hybridization has not been important, *Cyprinodon atrorus* and *C. bifasciatus* would be distinguishable at the mitochondrial and nuclear gene levels and patterns of variation in each species would independently reflect the influence of hydrogeographic history. Because mitochondrial genes evolve faster, they would be more likely than nuclear genes to show divisions across the major drainage systems. Findings from this study have important implications for understanding the hydrogeographic and hybridization history in these species, and demonstrate how hybridization can have important consequences for the evolution of animals.

Materials and Methods

Specimen Collection

We collected 212 *Cyprinodon atrorus* and 461 *C. bifasciatus* from seven and thirteen localities, respectively, representing the known distribution of both species in the Cuatro Cienegas basin, Mexico (Fig. 1). Collection localities for *C. atrorus* were: San Marcos vicinity, Laguna Grande, Laguna Salada, marshes associated with Canal Tio Julio (MJC20), Las Playitas/Charcos Prietos, Los Gatos, and Antiguos Mineros. *Cyprinodon bifasciatus* were collected from Poza Churince, La Becerra, Rio Garabatal, Juan Santos, Mojarral Oeste, Mojarral Este, Puente Orosco, Puente Dos Cuatas, an unnamed river near the origin of Canal Tio Julio (MJC 26), Escobeda, "Escobedita" (a small spring system ~0.35km N-NW of Escobeda), Tio Candido, and Los Hundidos. Specimens were preserved in 95% ethanol and whole genomic DNA was extracted from muscle tissue using the proteinase-K/phenol-chloroform as described in Tibbets and Dowling (1996).

SSCP Analyses

Mitochondrial (cyt*b*) and nuclear (CK-A7, RAG-1, and TPI-B4) gene sequence variation within and between populations and species was characterized through analysis of single-stranded conformational polymorphisms (SSCPs - Glavac and Dean 1993; Takeda et al. 1995) using α-^{32}P-dATP (Cetus-Perkin Elmer Corp) labeled DNA fragments. For cyt*b*, the SSCP primers LC_{Gila} (5' GCATCATTCTTCATCTGTAT 3'; Dowling unpublished) and HD (5' GGGTTGTTTGATCCTGTTTCGT 3'; Schmidt et al. 1998) were used to amplify a 312 bp segment of the gene under the following PCR

conditions: (94°C 1min; 48°C 1min; 72°C 2min; 25 cycles). For CK-A7, the primers CK-F1 (5'-CCCAAGTTYGAGGAGATCCTGAC-3') and CK-R1 (5'-CCGTCGACGACCAGCTGCACCTG-3') (Quattro and Jones 1999) were used to amplify a 282 bp fragment using the following PCR conditions: (94°C 30 sec; 60°C 30sec; 72°C 30sec; 30 cycles). For RAG-1, the primers Rag-B (5'-TTGGAAGTGTAGAGCCAGTGGT-3') and RAG-Cpup (5'-ATGGCGTGCTACCTGTCC-3'; Carson unpublished) were used to amplify a 355 bp fragment using the following PCR conditions: (94°C 30 sec; 55°C 30sec; 72°C 30sec; 30 cycles). For TPI-B4, the primers TPI-B4F2 (5'-GCATYGGGGAGAAGCTRGAT-3') and TPI-B4R1 (5'-AGAACCACYTTRCTCCAGTC-3') (Merritt and Quattro 2001) were used to amplify a 213 bp fragment using the following PCR conditions: (94°C 30 sec; 55°C 30sec; 72°C 30sec; 35 cycles). Polymerase chain reactions (PCR) were run in five uL total volume per sample. When amplification was completed, four uLs of stop solution (bromophenol blue and xylene cyanol) were added to each PCR product, denatured at 95°C for 3 minutes, and then placed on ice. SSCP gels were then run at room temperature for 18 hours at 6W on six percent (cyt*b* and RAG-1) or eight percent (CK-A7 and TPI-B4) acrylamide gels (37.5:1 acrylamide:bis-acrylamide, 1X TBE). Gels were dried and exposed to Kodak XAR film for 12-36 hours. Ambiguous haplotypes or haplotypes that were difficult to score across the gel were re-run on additional gels (or sequenced) until all haplotypes were resolved.

For RAG-1, two common alleles were present in *Cyprinodon bifasciatus* populations, but were difficult to discriminate on SSCP gels, though they were easily

distinguishable from *C. atrorus*-specific alleles. These two *C. bifasciatus* alleles were counted as a single allele. Although this underestimates genetic variation at the RAG-1 locus in *C. bifasciatus*, it does not change overall results of the survey (i.e., genetic differences between *C. atrorus* and *C. bifasciatus*). However, exclusion of this cryptic allele may have reduced resolution of population genetic structure within *C. bifasciatus* at this locus.

Sequencing

At least one representative of each SSCP variant per gel was sequenced using an ABI 377 automated sequencer. Amplification parameters to generate PCR products for sequencing the same as those used in SSCP except α-^{32}P-dATP was not used and reaction volumes were larger (25 ul). The cyt*b* primers LA (5' GTGACTTGAAAAACCACCGTTG 3' Schmidt et al. 1998) and HD (Schmidt et al. 1998) were used to amplify (94°C 1min; 48°C 1min; 72°C 2min; 25 cycles) the first half of the gene, including the 312 bp SSCP fragment. The primers LD_{rbs} (5' ACCCTAACACGATTCTTTGC 3'; Dowling unpublished) and HA (5' CAACGATCTCCGGTTTACAAGAC 3'; Schmidt et al. 1998) were used to amplify the remainder of the gene for each confirmed haplotypic variant, and resulting products sequenced with the LD primer to obtain the remaining portion of the gene. For CK-A7, RAG-1, and TPI-B4, PCR protocol for sequencing was identical to that of SSCP and allelic variants were sequenced in both directions using SSCP primers. All sequences were aligned by eye in MacDNAsis (Hitachi Corporation 1996).

Population Genetic Analysis

To place mitochondrial and nuclear gene variation in a phylogenetic context, maximum parsimony in PAUP (Swofford 1998) was used to determine relationships among haplotypes (cyt*b*) and nuclear alleles (CK-A7, RAG-1, and TPI-B-4). For cyt*b* and all three nuclear genes, Arlequin (Schneider et al. 2000) was used to estimate allele frequencies and test for Hardy-Weinberg equilibrium for nuclear loci and linkage disequilibrium (Schneider et al. 2000). P-values for Hardy-Weinberg and linkage disequilibrium analyses were corrected for multiple comparisons using the Bonferroni correction (Sokal and Rohlf 1995). AMOVA (Excoffier et al. 1992) in Arlequin (Schneider et al. 2000) was used to quantify population genetic structure for cyt*b*, CK-A7, RAG-1, and TPI-B4, and to calculate population genetic parameters (e.g., heterozygosity, nucleotide diversity, and nucleotide polymorphism.). Geographic structure of mitochondrial SSCP haplotypes was also examined using nested clade analysis (reviewed in Templeton 2001). TCS 1.18 (Clement et al. 2000) was used to determine clade structure and GeoDis 2.0 (Posada et al. 2000) was used to test for clade significance.

Results and Discussion

Nuclear Gene Variation

The distribution of genetic variation in Cuatro Cienegas *Cyprinodon* shows a complex interplay between species, hydrogeography, and hybridization. At the nuclear gene level, standard measures of genetic variation in *Cyprinodon atrorus* and *C.*

bifasciatus indicated that allelic variation is generally low in both species (Tables 1-3). In *Cyprinodon atrorus*, CK-A7 was most variable, segregating for three alleles typical of the species (cA1-cA3; Figure 2), with a single individual from Laguna Salada carrying an allele typical of *C. bifasciatus* (cB1; Table 4). At this locus, cienega and playa lake systems associated with the central basin (Laguna Salada, Las Playitas, Los Gatos, and cienegas associated with the origin of Canal Tio Julio (MJC 20) contained more variation than small or geographically restricted populations (Antiguos Mineros, Laguna Grande, and San Marcos). This pattern may indicate previously large population sizes of *C. atrorus* in the once expansive marsh and laguna habitats that historically characterized the central basin, and may also be indicative of the old age of these systems. At RAG-1, most populations of *C. atrorus* were fixed for a single allele (rA1; Figure 3) characteristic of the species, though three populations also carried a low frequency of an allele typical of *C. bifasciatus* (rB1; Table 5; Figure 3). Similarly, at TPI-B4 *C. atrorus* populations were generally fixed for a typical *C. atrorus* allele (tA1; Figure 4), although three populations carried a low frequency of the typical *C. bifasciatus* allele (tB1; Table 6; Figure 4) at this locus. Patterns observed at RAG-1 and TPI-B4 possibly reflect the boom and bust cycles characteristic of *C. atrorus* (Minckley 1969), with a limited amount of introgressive hybridization from *C. bifasciatus* (see below).

In *Cyprinodon bifasciatus*, CK-A7 showed the greatest variation (12 alleles), followed by RAG-1 (four alleles) then TPI-B4 (three alleles) (Tables 4-6). At CK-A7, most populations were fixed or nearly fixed for the common *C. bifasciatus* allele (cB1; Table 4; Figure 2), though presence of the common *C. atrorus* allele (cA1) was found in

four populations, while four populations also segregated at low frequency for alleles unique to *C. bifasciatus* (Table 4). At CK-A7, variation in *C. bifasciatus* was highest in Juan Santos and La Becerra/Rio Garabatal. Such variation is not surprising, as these populations are either presently (Juan Santos) or were historically large (Becerra/Garabatal) prior to drainage that followed canal construction in 1964. However, a surprising finding was that the expansive Rio Mesquites system, probably representing the numerically largest population of *C. bifasciatus* in the basin, showed no variation at the CK-A7 locus. Minimal variation in this system may indicate that the Rio Mesquites population is either younger than populations in the western basin (Juan Santos and Becerra/ Rio Garabatal) or has suffered an historical population bottleneck. The remaining smaller, isolated populations of *C. bifasciatus*, such as Escobeda, Los Hundidos, Poza Churince, and Tio Candido, contained no variation at CK-A7. Minimal variation in these populations is likely due to their relatively small population sizes and isolation from other systems.

At the remaining loci, RAG-1 and TPI-B4, variation in *Cyprinodon bifasciatus* was more limited. At RAG-1 (Table 5), most populations were fixed for the common *C. bifasciatus* allele (rB1; Figure 3), with minor variation contributed by rare alleles unique to *C. bifasciatus* (rB2 and rB3), and a low frequency of an allele typical of *C. atrorus* (rA1). Variation at RAG-1 was highest in Juan Santos, segregating for a common allele as well as an allele unique to this system. However, interpretation of variation at RAG-1 was confounded by one *C. bifasciatus* allele (rB2; Figure 3) that could not be reliably differentiated from the common *C. bifasciatus* allele (rB1) on SSCP gels. Thus, RAG-1

variation measured in *C. bifasciatus* populations almost certainly represents an underestimate of variation at this locus, and therefore may have reduced resolution of population genetic structure at this locus within *C. bifasciatus*. Nonetheless, all RAG-1 alleles characteristic of *C. bifasciatus* were easily distinguishable from the typical *C. atrorus* allele on SSCP gels and in nucleotide sequence. At TPI-B4 there were only three alleles in *C. bifasciatus* (Table 6). One of these alleles (tB1; Table 6; Figure 4) predominated, one was rare (tB2), and one (tA1) likely represented an introgressed allele from nearby *C. atrorus* populations (see below).

There was clear differentiation between *Cyprinodon atrorus* and *C. bifasciatus* (Tables 4-6; Figures 2-4) at the nuclear gene level. Minimum nucleotide differences between alleles typical of *C. atrorus* and *C. bifasciatus* varied from one in CK-A7, three in RAG-1, and two in TPI-B4 (Figures 2-4), with all changes represented by nucleotide substitutions. Most variation in CK-A7 (F_{CT}= 0.9283; P= 0.000), RAG-1 (F_{CT}= 0.9595; P= 0.000), and TPI-B4 (F_{CT}= 0.9871; P= 0.000) was partitioned among species rather than by geography. Variation among populations within species was low in TPI-B4 (F_{SC}=0.0002; P= 0.000), moderate in CK-A7 (F_{SC}= 0.1006; P= 0.000), and high in RAG-1 (F_{SC}= 0.4902; P= 0.000), with the elevated value at RAG-1 generated by a high frequency (69%) of a unique allele (rB3) at Juan Santos. Populations were generally in Hardy-Weinberg equilibrium at each locus (Table 7) and linkage equilibrium between loci (Table 8).

Evidence for limited gene exchange between *Cyprinodon atrorus* and *C. bifasciatus* occurred in nine surveyed populations (Tables 4-6): La Becerra, an unnamed

river associated with the origin of Canal Tio Julio (MJC 26), Puente Dos Cuatas, Puente Orosco, San Marcos vicinity, Laguna Grande, Laguna Salada, and cienegas associated with the origin of Canal Tio Julio (MJC20). In La Becerra, introgression of *C. atrorus* alleles into *C. bifasciatus* likely occurred with former contact between these species prior to substantial draining of this system in late 1964. In the river associated with the origin of Canal Tio Julio there is a known hybrid zone between *C. bifasciatus* and *C. atrorus* (Carson pers. obs.). Similarly, Puente Orosco is just upstream (<0.5km) of a peripheral *C. atrorus* population and limited hybridization between *C. atrorus* and *C. bifasciatus* has been observed in this region (Carson pers. obs.). Puente Orosco is also ~1 km upstream of Puente Dos Cuatas, a site that Minckley (pers. comm.) considered to contain hybrids. A similar pattern is seen in *C. atrorus* populations from San Marcos vicinity, Laguna Grande, Laguna Salada, and cienegas associated with Canal Tio Julio. Laguna Grande is the terminal sink of the Rio Churince, a system in which hybridization between *C. atrorus* and *C. bifasciatus* is extensive. San Marcos is in close proximity to Laguna Grande, and in periods with higher water levels would easily communicate with the Rio Churince system. Laguna Salada is peripheral to the mid-reaches of the Rio Mesquites and *C. atrorus* from this site occasionally hybridize with *C. bifasciatus* (Carson pers. obs.). Finally, cienegas associated with the origin of Canal Tio Julio abut a known hybrid zone between *C. bifasciatus* and *C. atrorus* (Carson pers. obs.). Together, these patterns suggest that sharing of alleles between *C. atrorus* and *C. bifasciatus* at these localities represents minor introgression (1-5%) associated with the mosaic structuring of habitats within Cuatro Cienegas rather than ancestral polymorphism.

Because CK-A7, RAG-1, and TPI-B4 clearly differentiate *C. atrorus* and *C. bifasciatus*, these loci are therefore useful for identification and study of hybridization between these species.

Mitochondrial Variation

Surveys of variation in a mitochondrial gene (cyt*b*) identified 10 and 15 haplotypes in *Cyprinodon atrorus* and *C. bifasciatus*, respectively. Sequence divergence was low (< 1%) between any pair of haplotypes; however, only four were shared between species (Table 9; Figure 5). In *C. atrorus* most variation (Table 9) was concentrated in the Los Gatos population, which resides in a large, stable marsh and laguna complex, whereas other *C. atrorus* populations were fixed or nearly fixed for single haplotypes (Table 9). Standard measures of population genetic variation (Table 10) showed that *C. atrorus* populations had an average of 2.3 haplotypes per population (range 1 to 8) and gene diversity of 0.15 (range 0.00 to 0.51). Estimated nucleotide diversity in *C. atrorus* was 0.001 (range 0.000 to 0.004) and the average pairwise comparison revealed 0.32 differences (range 0.00 to 1.30). Theta ranged from 0.00 to 2.09 (average 0.44). This overall pattern is similar to that found in the nuclear CK-A7, where central basin *C. atrorus* populations had the highest level of variation. As such, this pattern may indicate Los Gatos to be a major source population for genetic variation in *C. atrorus*.

In *C. bifasciatus*, standard population genetic measurements (Table 10) showed that this species had an average of three haplotypes per population (range 1-7). Most of this variation was harbored in the Rio Mesquites populations (Mojarral Este and Oeste,

Puente Dos Cuatas, and Puente Orosco; Table 9), with three other populations (Escobeda, MJC 26, and Rio Garabatal) having low to moderate variation (2-4 haplotypes). The remaining six populations of *C. bifasciatus* were fixed for a single haplotype. Gene diversity in *C. bifasciatus* ranged from 0.00 to 0.80 (average 0.32), whereas nucleotide diversity ranged from 0.000 to 0.006 (average 0.002) and the average pairwise comparison yielded 0.67 differences (range 0.00 to 1.78). Theta averaged 0.65 (range 0.00 to 1.69). This pattern is in contrast to that found in nuclear genes (CK-A7 and RAG-1), which indicated La Becerra, Rio Garabatal, and Juan Santos to have the highest variation and the Rio Mesquites to have minimal variation. Thus, the numerically large population of Rio Mesquites has retained a high level of mitochondrial diversity despite its low level of nuclear gene variation. Reasons for this are not clear, but if the Rio Mesquites population is younger than those from La Becerra/ Rio Garabatal and Juan Santos this might be expected, as the Rio Mesquites draws from many peripheral source populations that may have accumulated and maintained mitochondrial variation, while nuclear gene variation in these populations might have remained low due to insufficient time to accumulate new mutations. In addition, there is evidence that the Rio Mesquites population is composed of divergent sub-units (see below).

Mitochondrial Replacement

In contrast to nuclear genes, mitochondrial variation was not structured according to species (F_{CT}= 0.0895; P=0.0782), but was significantly partitioned among populations within species (F_{SC}= 0.7270; P= 0.000). A follow-up AMOVA analysis was used to

determine if genetic variation within and between species was distributed according to geography, specifically Minckley's (1969) hypothesis of surficial drainage relationships in the basin: 1) "Western" contained Laguna Grande, San Marcos, and Poza Churince, Becerra, Rio Garabatal, and Juan Santos; 2) "Rio Mesquites" contained Mojarral East, Mojarral West, Puente Orosco, and Puente Dos Cuatas; 3) "Rio Puente Chiquita-Las Playitas" was comprised of cienegas associated with the origin of Canal Tio Julio (MJC 20), an unnamed river associated with the origin of Canal Tio Julio (MJC 26), Laguna Salada, Las Playitas, and Los Gatos; 4) "Southeast" populations included Escobeda, Escobedita, Los Hundidos, and Tio Candido; and 5) "Santa Tecla," contained only Antiguos Mineros. This analysis of population genetic variation with respect to hydrogeography revealed a significant geographic component to population genetic structure at the mitochondrial level (F_{ST}= 0.7670; P=0.000), with variation partitioned among drainage regions (F_{CT}= 0.5649; P=0.000) and among populations within regions (F_{SC}= 0.4646; P= 0.000). Because *Cyprinodon atrorus* and *C. bifasciatus* are not closely related within the genus (Miller 1968, Echelle and Echelle 1998, Echelle et al 2005), this finding, along with mitochondrial similarity and nuclear gene dissimilarity between these species, suggests introgressive hybridization has led to the complete replacement of one species mitochondrial genome by that of the other. Contrasts of allozyme and mitochondrial phylogenies of the genus (Echelle and Echelle 1998, Echelle et al. 2005), as well as morphological evidence (Miller 1968; Lozano et al 1993), indicate that the *C. bifasciatus* mitochondrial genome has been replaced by that of *C. atrorus*. Although there have been demonstrations of mitochondrial replacement in parts of species' ranges

(Ferris et al. 1983, Tegelstrom 1987, Dowling et al 1989, Dowling and Hoeh 1991, Bernatchez et al. 1995, Wilson and Bernatchez 1998), to our knowledge this is the first demonstration of complete replacement of an entire species' mitochondrial genome. There are other possible cases in pupfishes, as indicated by conflicting phylogenetic hypotheses from allozyme and mtDNA (Echelle et al. 2005), but these require further corroboration.

*Hydrogeographic Hypotheses and Cyt*b *Population Genetic Structure*

Following mitochondrial replacement there has apparently been diversification into two primary lineages (Figure 5), "A" and "B," which are subdivided into three major nested clades (2-1, 2-2, and 2-3; Figure 6). Lineages and nested clades, in turn, were roughly partitioned across the major hydrogeographic regions as defined by Minckley (1969) rather than by species. In the western basin there was partial correspondence to Minckley's description of two separate drainage systems, the Rio Churince and La Becerra/Rio Garabatal/Juan Santos. Here, two haplotypes were found, "A" and "I" ("A" lineage). The "A" haplotype was shared by *Cyprinodon atrorus* from the Laguna Grande/San Marcos populations and *C. bifasciatus* from Poza Churince and La Becerra/Rio Garabatal. While there is no present surface connection between La Becerra/Rio Garabatal and Poza Churince/Laguna Grande/San Marcos, our results suggest that there has probably been relatively recent contact between these two major systems. This connection could have occurred through *C. atrorus*, which inhabits marshes and lagunas on the barrial and would have easily communicated between the two

systems during periods of higher water level, perhaps during the period that predated the Holocene. In contrast, the isolated Juan Santos site was differentiated from other western basin systems, as it was fixed for a unique haplotype, "I," that differed from the "A" haplotype by four mutations. Fixation of the unique "I" haplotype in Juan Santos, combined with a high frequency of a unique allele at RAG-1 and substantial variation in CK-A7, makes this population a high priority for conservation.

The pattern of variation found in the central and southeastern basin was also consistent with Minckley's hypothesis. In the central basin, *Cyprinodon atrorus* from Los Gatos showed the greatest mitochondrial diversity with eight haplotypes (Table 9). This result is not surprising, as Los Gatos is an extensive marsh/laguna system that harbors a large, relatively stable *C. atrorus* population. Other populations of *C. atrorus* in the central basin showed evidence of past connection to Los Gatos through a high or fixed frequency of the common "B" haplotype ("B" lineage). Nested clade analysis suggests that the geographical distribution of haplotypes in this region, represented by clade 1-1 (Figure 6), could be explained by restricted gene flow with isolation by distance. Although these populations are not connected today, it is likely that they would have been in contact during pluvial periods predating the Holocene. In addition, human activity (canal construction) may have recently diminished connectivity between these populations through a general lowering of the water table in the basin (Minckley 1969). Thus, there is strong evidence that *C. atrorus* from the central basin (Las Playitas, Laguna Salada, Los Gatos, and cienegas associates with the origin of Canal Tio Julio) inhabits a historically broadly interconnected drainage region.

In the far southeast of the basin, *Cyprinodon atrorus* from Antiguos Mineros shared with *C. atrorus* from Los Gatos the "B" (30% and 69%, respectively) and "Q" (70% and 2.4%, respectively) haplotypes ("B" lineage), suggesting former connection between these localities. However, the high frequency of the "Q" haplotype at Antiguos Mineros, and its rarity at Los Gatos, suggests that this population has been isolated from Los Gatos for a considerable period. This interpretation is agrees with Minckley's hypothesis that Santa Tecla (Antiguos Mineros) represents a distinct hydrogeographic unit. In fact, nested clade analysis suggests that the geographical distribution of haplotypes in clade 2-1 (Figure 6) is best explained by restricted gene flow with some long distance dispersal. This result is plausible, as during times of higher water level in the basin *C. atrorus* from Los Gatos could easily have expanded its range across the barrial via large lagunas and cienegas that would have likely existed between these sites.

In further support of Minckley's hypothesis, *Cyprinodon bifasciatus* from populations in the southeastern basin (Escobeda, Los Hundidos, and Tio Candido) all appear more closely related to each other than to populations from other regions. This is supported by fixation of the "G" haplotype in Los Hundidos and Tio Candido, and a high frequency of this haplotype in Escobeda (~74%). The "G" haplotype is in turn more closely related to the "B" haplotype ("B" lineage) predominant in *C. atrorus* from the central and southeastern basin than the haplotypes that predominate in *C. bifasciatus* from the middle/upper Rio Mesquites and western basin ("A" lineage). Nested clade analysis revealed that the geographic distribution of haplotypes from clade 2-2 (Figure 6) is best explained by past fragmentation. This is also supported by apparent past surface

connection between Escobeda, Escobedita, Tio Candido, and Los Hundidos, which is obvious on satellite images that show a remnant riverbed connecting these areas.

Presence of the "L" haplotype at Escobeda and Escobedita suggests that these populations may have had a former connection to the Rio Mesquites, as this haplotype is more closely related to haplotypes from the upper Rio Mesquites and western basin ("A" lineage) than those from the southeastern portion of the basin ("B" lineage). The hypothesized connection between Escobeda/Escobedita and the lower Rio Mesquites is also supported by presence of a spring-deposited travertine field originating at Escobeda and running toward the lower Rio Mesquites. It is also possible that Escobeda gained contact with the Rio Mesquites through human mediation (Escobeda was also canalized in the late 1800's in the direction of the lower Rio Mesquites), though there is presently no surface connection between the two. However, because the "L" haplotype is fixed in the nearby, but isolated Escobedita site, human activity alone cannot explain the presence of the "L" haplotype in both Escobeda and Escobedita. This suggests that these sites sit at a pivotal position between the southeastern portion of the basin and the lower Rio Mesquites, and thus are a high priority for conservation.

Minckley considered the Rio Mesquites as a single unit; however, our results suggest that the upper and lower reaches of this system are divergent, with the upper system (Mojarral East and Mojarral West) mainly composed of haplotypes from lineage "A" and clade 2-3, and the lower segment (Puente Orosco and Puente Dos Cuatas) harboring haplotypes from lineages "A" and "B" and clades 2-1, 2-2, and 2-3. For example, *Cyprinodon bifasciatus* from the upper Rio Mesquites segregated in moderate

to high frequency for seven haplotypes (Table 9), four of which were not found in the lower Rio Mesquites populations. In turn, the lower Rio Mesquites populations harbored a high frequency of haplotypes not found in the upper Rio Mesquites, or found there at low frequency. For example, haplotypes typical of *C. atrorus* from the surrounding cienegas (Table 9), as well as the "G" haplotypes common in southeastern *C. bifasciatus* (Escobeda, Tio Candido, and Los Hundidos), were found at appreciable frequency in the lower Rio Mesquites. Similarly, the "L" haplotype that was fixed in Escobedita and common in Escobeda (18%) was also common in Puente Orosco (25%) and Puente Dos Cuatas (23%).

Two explanations can account for the pattern of *Cyprinodon bifasciatus* differentiation between the upper and lower reaches of the Rio Mesquites. First, lower reaches of the Rio Mesquites are thought to be separated from the upper Rio Mesquites by a large intervening marsh system that may serve as a partial barrier to gene flow between these river segments. Second, the upper Rio Mesquites populations of *C. bifasciatus* are not generally surrounded by *C. atrorus*, and thus hybridization and introgression of typical *C. atrorus* haplotypes into *C. bifasciatus* from the upper Rio Mesquites is very limited. Lower localities, however, are in close proximity to *C. atrorus* that is highly abundant in surrounding marshland. Thus, hybridization with *C. atrorus* likely explains the presence of common eastern *C. atrorus* haplotypes (B and E) in *C. bifasciatus* from the lower Rio Mesquites. In fact nuclear genes further support this argument as there was minor introgression of *C. atrorus* alleles into both Puente Dos Cuatas (at CK-A7, RAG-1, and TPI-B4) and Puente Orosco (at CK-A7). In addition,

there is also strong evidence that the Escobeda system formerly flowed into the Rio Mesquites near Puente Orosco and Puente Dos Cuatas, and therefore it is not surprising that these populations share haplotypes with the southeastern populations of *C. bifasciatus* (Escobeda, Escobedita, Tio Candido, and Low Hundidos).

A similar pattern was observed in *C. bifasciatus* from an unnamed river that flows into the origin of Canal Tio Julio. Here the *C. bifasciatus* population is thought to inhabit an isolated, unnamed river that is surrounded by marshes with large *C. atrorus* populations. This riverine system is also in close proximity to lower reaches of the Rio Mesquites. In this unnamed river system, the *C. bifasciatus* population had a high frequency (50 %) of the "B" haplotype (lineage "B"). This site also had a high frequency of the "G" haplotype (47%; lineage "B") that is common in nearby Puente Orosco (35%) and Puente Dos Cuatas (23%) and fixed in the remaining southeastern *C. bifasciatus* populations (Los Hundidos and Tio Candido). Thus, mitochondrial haplotypes in this unnamed river system appear to share a close affinity with those from central basin *C. atrorus* and southeastern *C. bifasciatus*.

Dynamics of Mitochondrial Replacement

What is surprising is that *Cyprinodon atrorus* from the central cienegas showed no evidence of mitochondrial introgression from surrounding *C. bifasciatus* populations. This overall asymmetry of mitochondrial gene flow into *C. bifasciatus* populations is similar to the asymmetrical introgression led to the complete replacement of *C. bifasciatus* mitochondrial genome. Whether this reflects dynamics of the original

mitochondrial replacement of *C. bifasciatus* genome is unknown. However, in other regions of the basin, such as La Becerra and Poza Churince, *C. bifasciatus* populations are fixed for an SSCP haplotype that is also fixed in a connected or nearby *C. atrorus* population. *Cyprinodon bifasciatus* populations in the basin that are isolated from *C. atrorus* generally are fixed for or segregate for alleles unique to *C. bifasciatus* (Table 7). These patterns suggest that when *C. bifasciatus* and *C. atrorus* come into contact, there is a predictable replacement of *C. bifasciatus* mitochondrial haplotypes by those of *C. atrorus*, with a cyclical pattern of isolation and divergence followed by re-contact and mitochondrial replacement. While the dynamics of such replacement remain unknown, the general pattern suggests a scenario in which *C. bifasciatus* mitochondrial genome was outnumbered and consequently replaced by that of *C. atrorus*. In this situation, hybridization would have led to a constant pulse of *C. atrorus* mitochondria towards *C. bifasciatus* populations, which in turn would have led replacement of *C. bifasciatus* mitochondrial genome. For example, such a scenario makes sense in light of mating behaviors of these species. In regions of contact, male *C. atrorus* and *C. bifasciatus* generally mate in shallow pools in which *C. atrorus* females are abundant. However, in areas where *C. atrorus* and *C. bifasciatus* ranges overlap, female *C. bifasciatus* usually congregate in schools away from shallows and into either laguna or riverine habitats. Thus, the direction of mitochondrial gene flow in hybrid populations is preferentially from *C. atrorus* females to hybrid offspring. Because *C. bifasciatus* is surrounded by numerically larger *C. atrorus* populations, mitochondrial replacement has potentially occurred due to the overwhelming number of *C. atrorus* in the system and the direction

of mating preferences within the hybrid zone. If such patterns have been a general trend throughout the history of contact between these species, then the original replacement of *C. bifasciatus* mitochondrial genome may also have been effected by mating site selection and a numeric bias towards *C. atrorus* mitochondria, rather than selection *for* the *C. atrorus* mitochondrial genome.

Conclusions

Results from this study suggest that both hydrogeography and hybridization have played critical roles in distribution of mitochondrial variation in Cuatro Cienegas *Cyprinodon*. At the nuclear gene level, these species are easily distinguishable, with allelic variation reflecting morphological species differences. Nuclear genes also suggest that the oldest *C. bifasciatus* populations occur in the western basin (Juan Santos and Becerra/Rio Garabatal), whereas the oldest *C. atrorus* populations occur in the central basin. Patterns at the mitochondrial level are in stark contrast to those observed at nuclear loci. At cyt*b*, an old hybridization event led to the complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus*. Subsequent diversification at the mitochondrial level has occurred with respect to hydrogeography that roughly corresponds to Minckley's (1969) seven drainage hypothesis. There is also evidence that mitochondrial replacement acts in a cyclical manner, with isolation and divergence followed by re-contact between *C. atrorus* and *C. bifasciatus* that leads to mitochondrial replacement of *C. bifasciatus* haplotypes.

Numerous studies have used mitochondrial genes to assess population genetic history in a wide array of plants and animals. Such use of mitochondrial genes has been preferred due to the fast mutation rates of mitochondrial genes, their maternal inheritance, and the lack of recombination in this molecule. However, mitochondrial genes are also known to introgress more readily in hybridizing taxa than are nuclear genes, presumably because nuclear genes are under stronger selection and thus create complications when expressed in a foreign genetic background. Use of nuclear genes for population level studies, however, has generally been avoided because these genes usually mutate too slowly to allow for robust reconstruction of population genetic structure within species and comparison of genetic differences between closely related species. Nonetheless, inclusion of both mitochondrial and nuclear gene data sets can provide insights that would not be apparent using only one of these types of markers.

This study demonstrates that understanding population genetic history in some species requires inclusion of both mitochondrial and nuclear gene data sets. Sole reliance on mitochondrial genes in *Cyprinodon atrorus* and *C. bifasciatus* would have underestimated differences between these species and could have led to the erroneous conclusion that *C. atrorus* and *C. bifasciatus* are not distinct taxa. However, inclusion of nuclear genes demonstrated clear genetic differences between these species, which is in keeping with substantial morphological distinctiveness of *C. atrorus* and *C. bifasciatus* (Miller 1968). Mitochondrial cyt*b* on the other hand proved useful in assessing hydrogeographic relationships within the basin, even though *C. bifasciatus* mitochondrial genome has been replaced by that of *C. atrorus*. Thus, this study suggests that inclusion

of both nuclear and mitochondrial DNA in other species can provide an effective means for gaining a comprehensive understanding of the population genetic history.

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Figure 1. Map of the Cuatro Cienegas basin, Coahuila, Mexico. Numbers indicate major drainages hypothesized by Minckley (1969): 1) Rio Churince; 2) La Becerra/Rio Garabatal/Juan Santos; 3) Rio Mesquites; 4) Rio Puente Chiquita; 5) Escobeda/Tio Candido/Los Hundidos; 6) Santa Tecla; and 7) Rio Salado de los Nadadores. Letters represent collection sites for *Cyprinodon atrorus* and *C. bifasciatus* A) San Marcos Vicinity; B) Poza Churince; C) Laguna Grande; D) Becerra; E) Rio Garabatal; F) Juan Santos; G) Mojarral Oeste; H) Mojarral Este; I) Puente Orosco; J) Puente Dos Cuatas; K) Laguna Salada; L) unnamed river associated with the origin of Canal Tio Julio; M) Cienegas associated with the origin of Canal Tio Julio; N) Las Playitas; O) Los Gatos; P) Antiguos Mineros; Q) Escobedita; R) Escobeda; S) Tio Candido; and T) Los Hundidos. Figure modified from Minckley (1969).

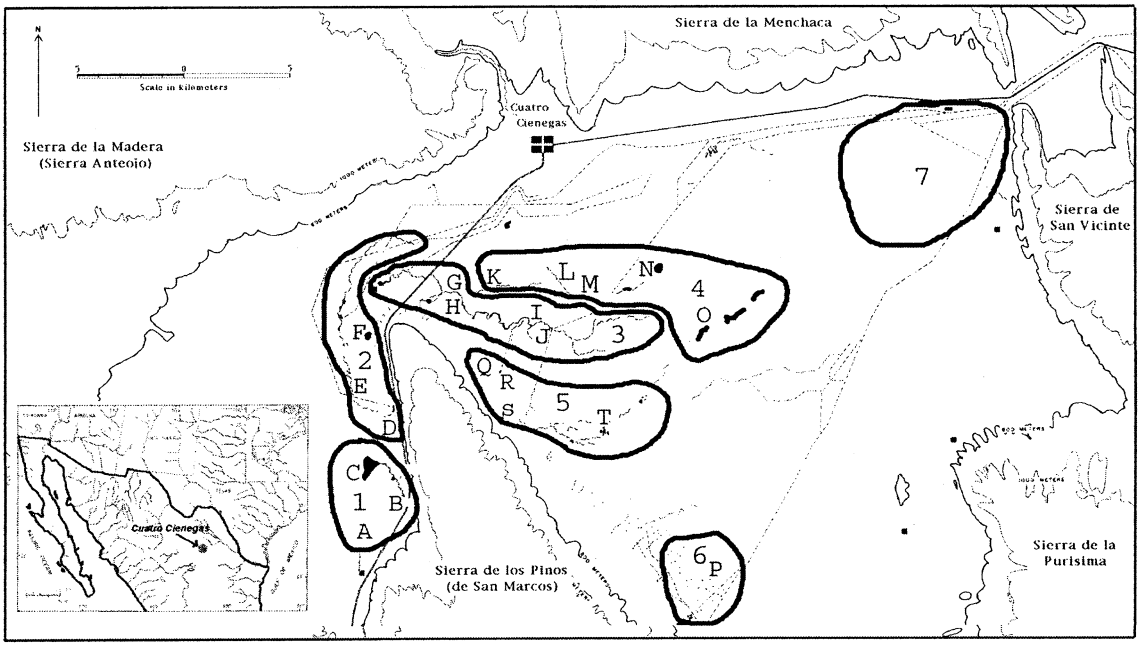

Sierra de la Menchaca
Cuatro Cienegas
Sierra de la Madera
(Sierra Anteojo)
Sierra de San Vicinte
Sierra de los Pinos
(de San Marcos)
Sierra de la Purisima
Cuatro Cienegas
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
1
2
3
4
5
6
7

Figure 2. Maximum parsimony tree obtained from analysis of sequence from a portion of the nuclear CK-A7 gene (length = 10 steps, CI= 0.600, RI = 0.714, uninformative characters excluded from the analysis but included in branch lengths of the figure). Alleles cA1-cA4 represent alleles typically associated with *Cyprinodon atrorus*. Alleles labeled cB1-cB10 represent alleles typical of *C. bifasciatus*. Numbers represent number of steps.

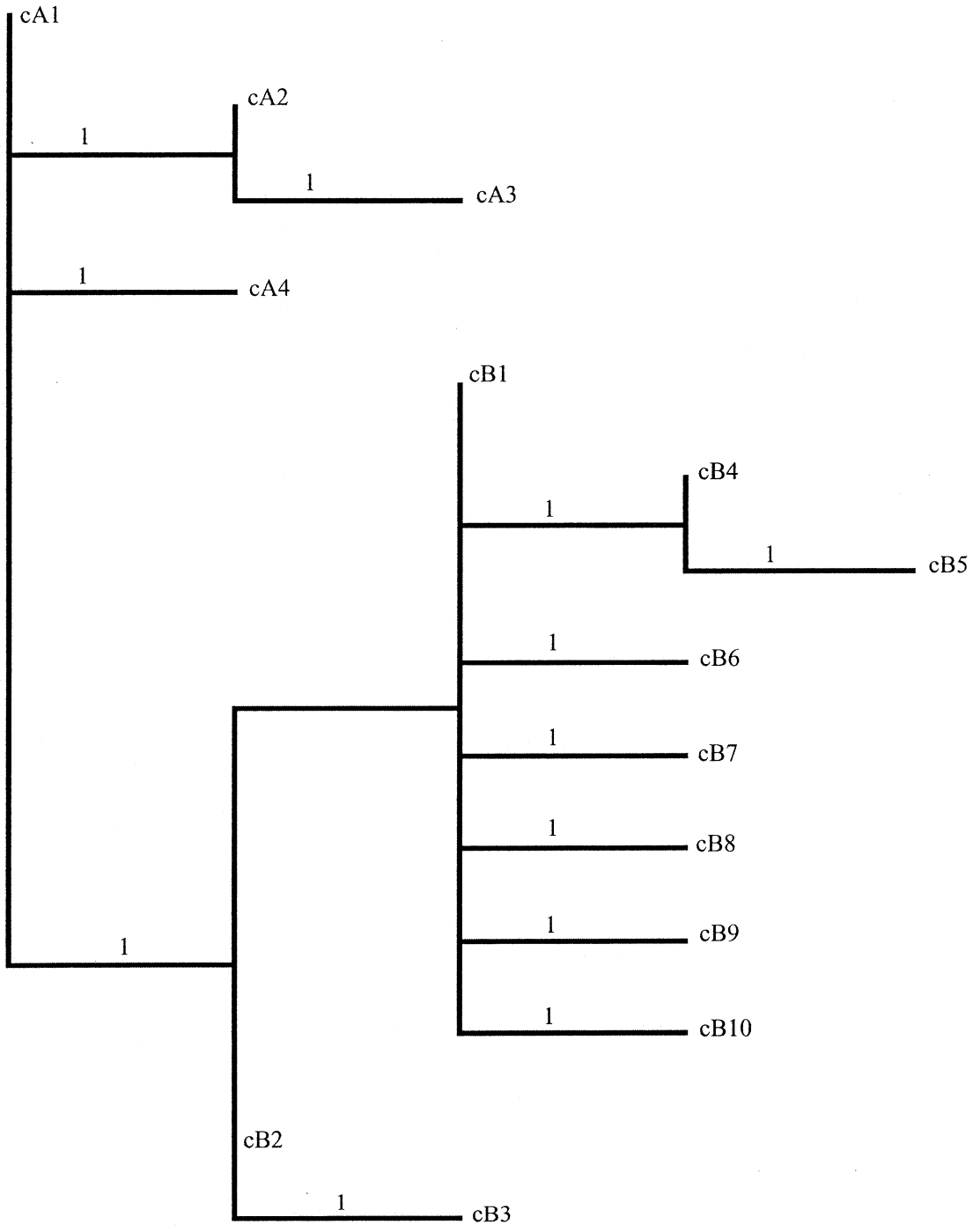

cA1
cA2
1
1
cA3
1
cA4
cB1
cB4
1
1
cB5
1
cB6
1
cB7
1
cB8
1
cB9
1
cB10
1
cB2
1
cB3

Figure 3. Maximum parsimony tree obtained from analysis of sequence from a portion of the nuclear RAG-1 gene (length = 5 steps, CI= 1.00, RI = 0/0, uninformative characters excluded from the analysis but included in branch lengths of the figure). Alleles cA1-cA4 represent alleles the typify *Cyprinodon atrorus*. Allele rA1 represents the typical *Cyprinodon atrorus* allele and alleles rB1-rB3 represent typical *C. bifasciatus* alleles. Numbers represent number of steps.

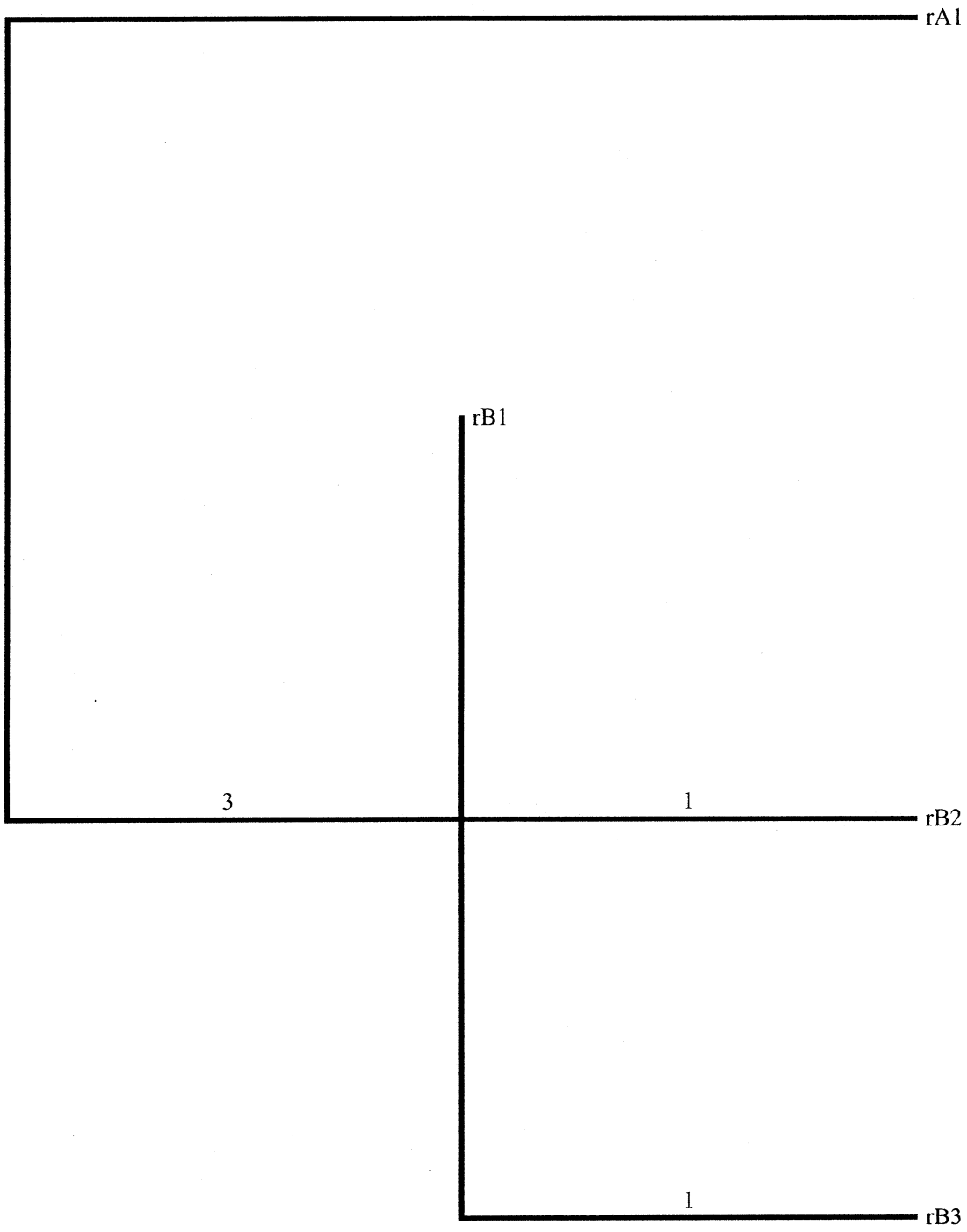

rA1
rB1
3
1
rB2
1
rB3

Figure 4. Maximum parsimony tree for TPI-B4. Allele tA1 represents the typical *Cyprinodon atrorus* allele. Alleles tB1and tB2 represent typical *C. bifasciatus* alleles. Numbers represent number of steps.

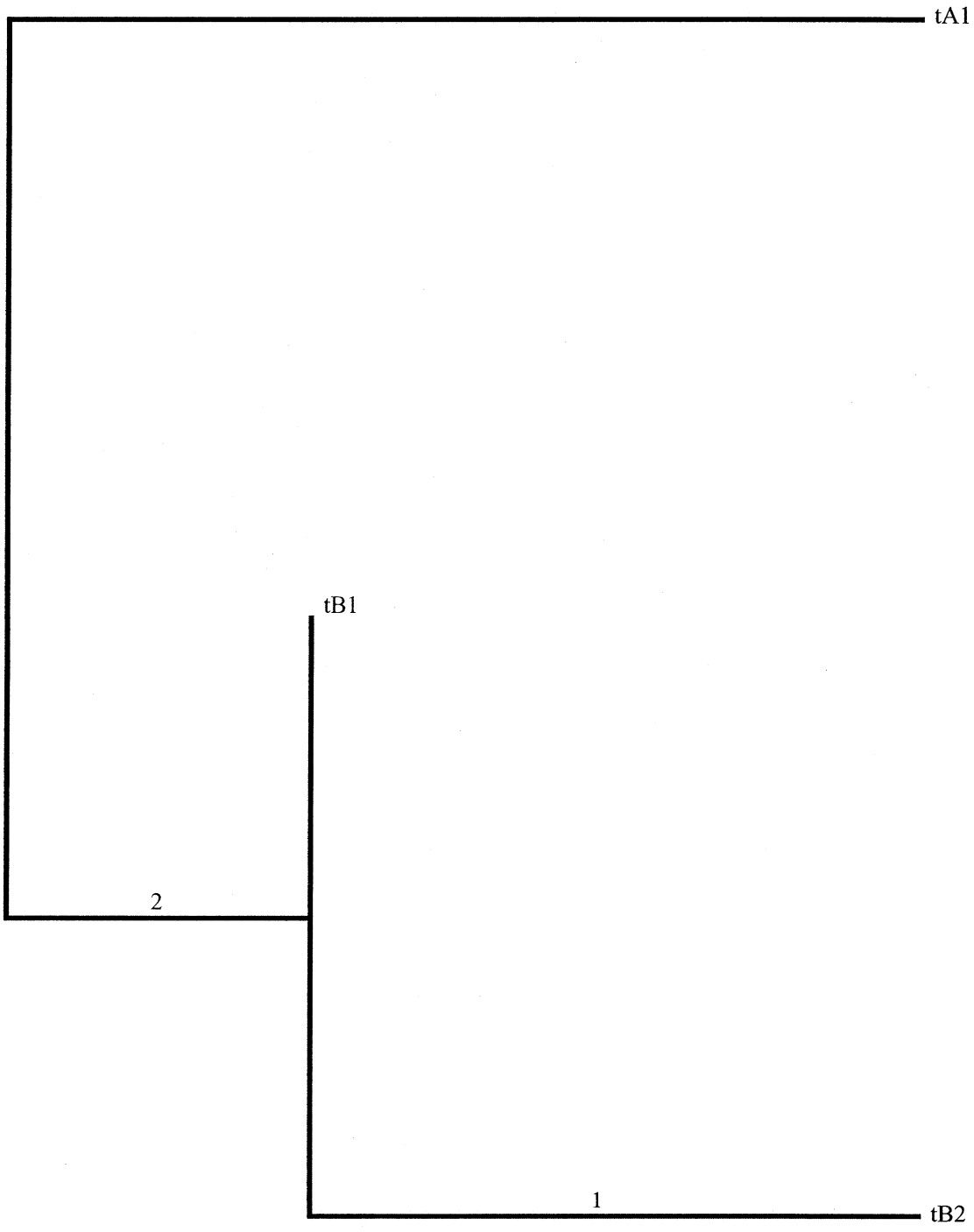

tA1
tB1
2
1
tB2

Figure 5. One of three maximum parsimony trees obtained from analysis of sequence from a portion of the mitochondrial cyt*b* gene (length = 70 steps, CI= 0.80, RI = 0.932, uninformative characters excluded from the analysis but included in branch lengths of the figure). The outgroup is represented by *Cyprinodon sp.* from the Rio Aguanaval, Mexico. Upper numbers represent number of steps, whereas lower numbers represent level of bootstrap support.

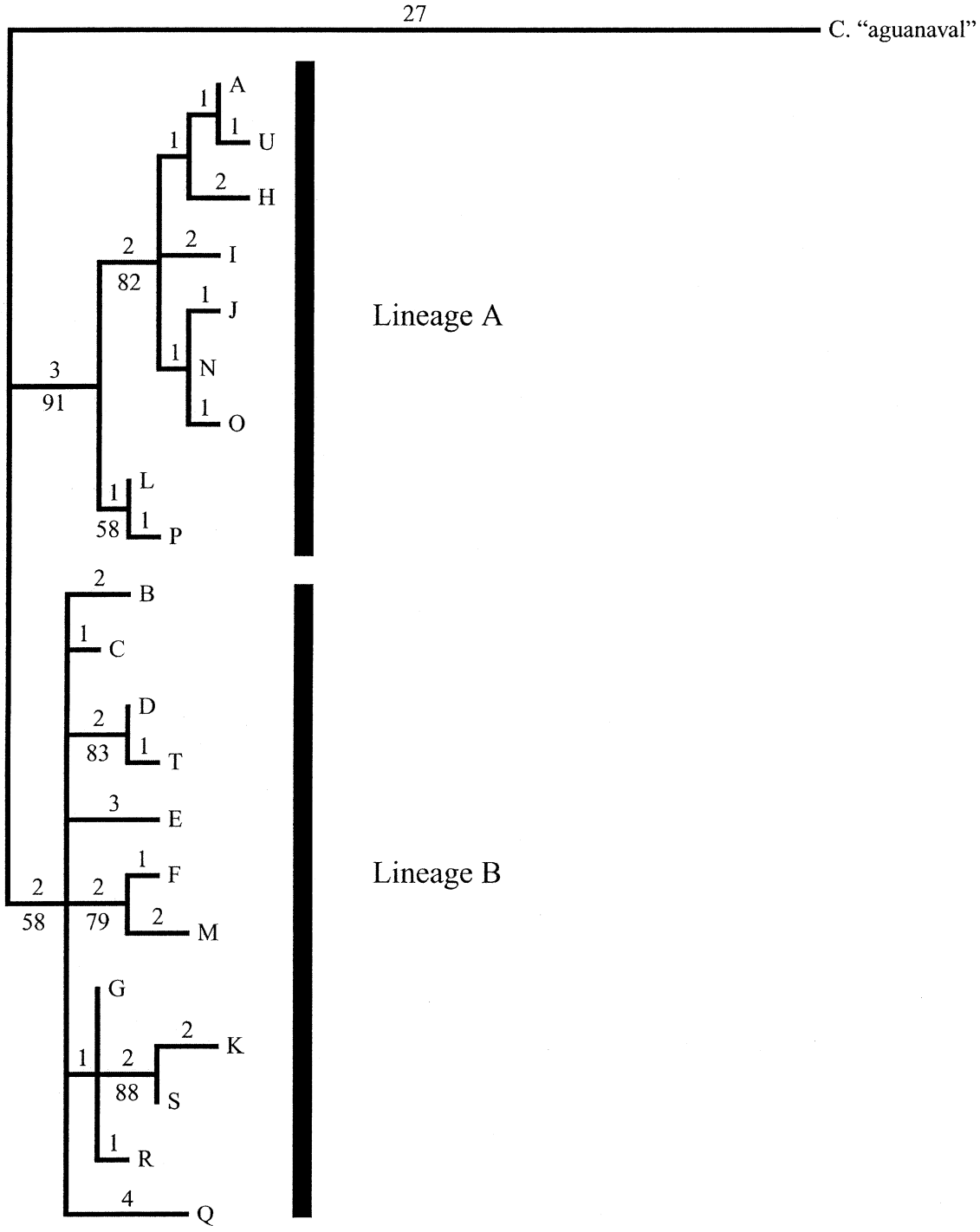

27
C. "aguanaval"
A
U
H
I
J
N
O
L
P
Lineage A
B
C
D
T
E
F
M
G
K
S
R
Q
Lineage B
1
2
3
4
82
91
58
83
79
88

Figure 6. Cyt*b* tree produced by TCS 1.18 for nested clade analysis of mitochondrial variation. Boxes represent nesting levels into nine one-step and three two-step clades. Letters denote mitochondrial haplotype as in Figure 5.

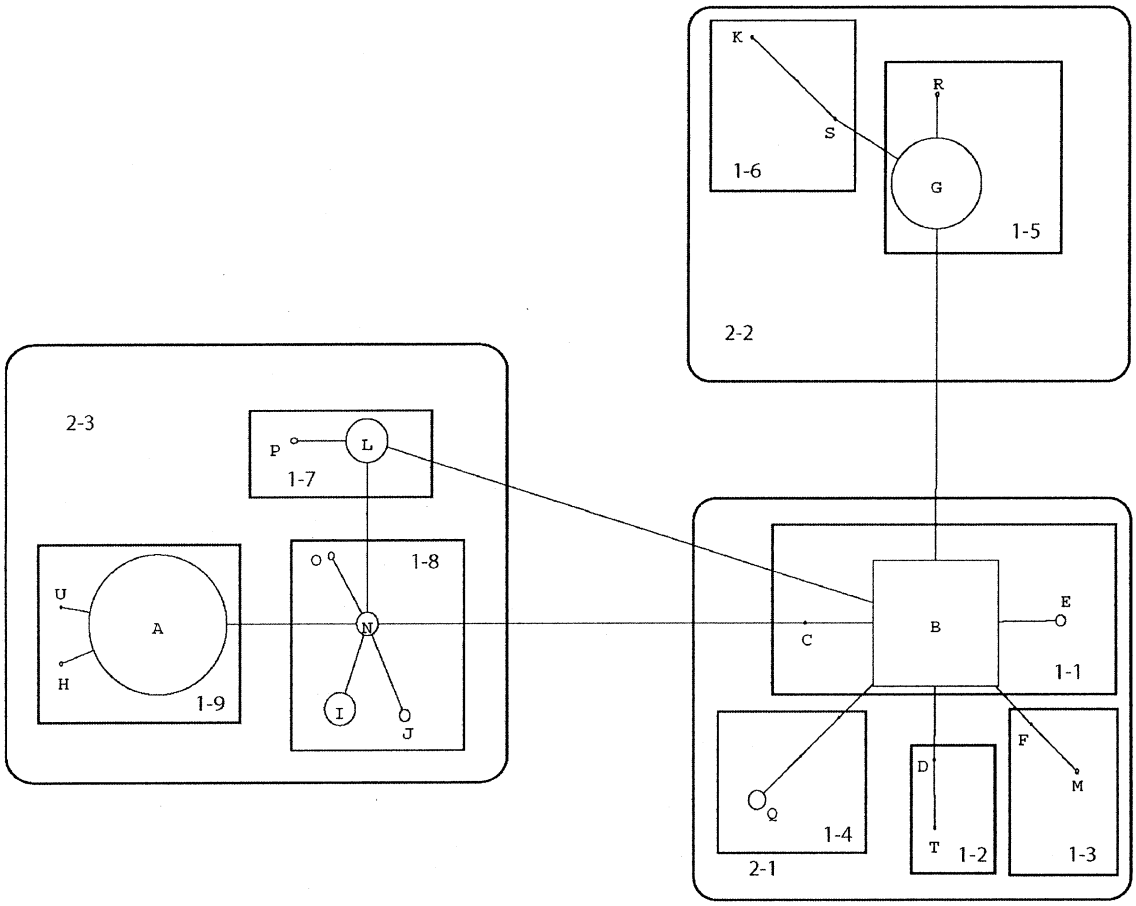

K
S
1-6
R
G
1-5
2-2
2-3
P
L
1-7
U
A
H
1-9
1-8
N
I
J
C
B
E
1-1
Q
1-4
2-1
D
T
1-2
F
M
1-3

Table 1. Standard measures of genetic variation in CK-A7 for each population (as labeled in Figure 1).

Table 1.
Standard Measures of Genetic Variation for Each Population at CK-A7

Locality	N	Number of Alleles	Theta	Gene Diversity	Mean Number of Pairwise Differences	Nucleotide Diversity
C. atrorus populations						
Antiguos Mineros	28	1	0 (0)	0 (0)	0 (0)	0 (0)
Laguna Grande	40	1	0 (0)	0 (0)	0 (0)	0 (0)
Laguna Salada	30	4	0.8578 (0.4716)	0.4847 (0.0477)	0.5540 (0.4611)	0.0024 (0.0022)
Las Playitas	30	3	0.4289 (0.3137)	0.0977 (0.0519)	0.0992 (0.1721)	0.0004 (0.0008)
Los Gatos	32	3	0.4230 (0.3091)	0.2009 (0.0632)	0.2045 (0.2550)	0.0009 (0.0012)
MJC 20	29	2	0.2160 (0.2160)	0.4217 (0.0505)	0.4229 (0.3899)	0.0018 (0.0018)
San Marcos	10	1	0 (0)	0 (0)	0 (0)	0 (0)
C. bifasciatus populations						
Becerra	37	7	1.0257 (0.5143)	0.2966 (0.0690)	0.3938 (0.3723)	0.0017 (0.0018)
Escobeda	37	1	0 (0)	0 (0)	0 (0)	0 (0)
Juan Santos	40	3	0.4038 (0.2943)	0.1421 (0.0513)	0.1441 (0.2097)	0.0006 (0.0010)
MJC 26	30	1	0.2145 (0.2145)	0.0333 (0.0320)	0.0334 (0.0979)	0.0001 (0.0005)
Mojarral Este	30	2	0 (0)	0 (0)	0 (0)	0 (0)
Mojarral Oeste	35	1	0.6226 (0.3823)	0.0567 (0.0381)	0.1423 (0.2085)	0.0006 (0.0010)
Los Hundidos	35	3	0 (0)	0 (0)	0 (0)	0 (0)
Poza Churince	39	1	0 (0)	0 (0)	0 (0)	0 (0)
Puente Dos Cuatas	59	2	0.3743 (0.2717)	0.0336 (0.0230)	0.0676 (0.1398)	0.0003 (0.0007)
Puente Orosco	39	2	0.6088 (0.3730)	0.0506 (0.0340)	0.1532 (0.2168)	0.0007 (0.0010)
Rio Garabatal	30	4	0.4289 (0.3137)	0.3701 (0.0727)	0.4161 (0.3860)	0.0018 (0.0018)
Tio Candido	30	1	0 (0)	0 (0)	0 (0)	0 (0)

Table 2. Standard measures of genetic variation in RAG-1 for each population (as labeled in Figure 1).

Table 2.
Standard Measures of Genetic Variation for Each Population at RAG-1

Locality	N	Number of Alleles	Theta	Gene Diversity	Mean Number of Pairwise Differences	Nucleotide Diversity
C. atrorus populations						
Antiguos Mineros	29	1	0 (0)	0 (0)	0 (0)	0 (0)
Laguna Grande	40	2	0.6057 (0.3709)	0.0250 (0.0242)	0.0750 (0.1481)	0.0003 (0.0006)
Laguna Salada	30	2	0.6433 (0.3965)	0.0655 (0.0433)	0.1966 (0.2496)	0.0007 (0.0010)
Las Playitas	30	1	0 (0)	0 (0)	0 (0)	0 (0)
Los Gatos	32	1	0 (0)	0 (0)	0 (0)	0 (0)
MJC 20	30	1	0 (0)	0 (0)	0 (0)	0 (0)
San Marcos	10	2	0.8456 (0.5403)	0.1000 (0.0880)	0.3000 (0.3263)	0.0011 (0.0013)
C. bifasciatus populations						
Becerra	31	2	0.2129 (0.2129)	0.0635 (0.0420)	0.0635 (0.1361)	0.0002 (0.0006)
Escobeda	39	1	0 (0)	0 (0)	0 (0)	0 (0)
Juan Santos	40	2	0.2019 (0.2019)	0.4351 (0.0396)	0.4351 (0.3953)	0.0016 (0.0016)
MJC 26	30	1	0 (0)	0 (0)	0 (0)	0 (0)
Mojarral Este	30	1	0 (0)	0 (0)	0 (0)	0 (0)
Mojarral Oeste	35	1	0 (0)	0 (0)	0 (0)	0 (0)
Los Hundidos	36	1	0 (0)	0 (0)	0 (0)	0 (0)
Poza Churince	39	1	0 (0)	0 (0)	0 (0)	0 (0)
Puente Dos Cuatas	59	2	0.5614 (0.3413)	0.0336 (0.0230)	0.1008 (0.1725)	0.0004 (0.0007)
Puente Orosco	37	1	0 (0)	0 (0)	0 (0)	0 (0)
Rio Garabatal	30	2	0.2145 (0.2145)	0.0966 (0.0507)	0.0966 (0.1698)	0.0004 (0.0007)
Tio Candido	30	1	0 (0)	0 (0)	0 (0)	0 (0)

Table 3. Standard measures of genetic variation in TPI-B4 for each population (as labeled in Figure 1).

Table 3.
Standard Measures of Genetic Variation for Each Population at TPI-B4

Locality	N	Number of Alleles	Theta	Gene Diversity	Mean Number of Pairwise Differences	Nucleotide Diversity
C. atrorus populations						
Antiguos Mineros	29	1	0 (0)	0 (0)	0 (0)	0 (0)
Laguna Grande	40	2	0.4038 (0.2943)	0.0494 (0.0332)	0.0987 (0.1711)	0.0006 (0.0011)
Laguna Salada	30	2	0.4289 (0.3137)	0.0333 (0.0320)	0.0667 (0.1397)	0.0004 (0.0009)
Las Playitas	30	1	0 (0)	0 (0)	0 (0)	0 (0)
Los Gatos	32	1	0 (0)	0 (0)	0 (0)	0 (0)
MJC 20	30	2	0.4289 (0.3137)	0.0333 (0.0320)	0.0667 (0.1397)	0.0004 (0.0009)
San Marcos	10	1	0 (0)	0 (0)	0 (0)	0 (0)
C. bifasciatus populations						
Becerra	36	2	0.2063 (0.2063)	0.0548 (0.0366)	0.0548 (0.1259)	0.0003 (0.0008)
Escobeda	38	1	0 (0)	0 (0)	0 (0)	0 (0)
Juan Santos	38	1	0 (0)	0 (0)	0 (0)	0 (0)
MJC 26	30	2	0.4354 (0.3187)	0.0357 (0.0341)	0.0714 (0.1449)	0.0004 (0.0009)
Mojarral Este	28	1	0 (0)	0 (0)	0 (0)	0 (0)
Mojarral Oeste	30	1	0 (0)	0 (0)	0 (0)	0 (0)
Los Hundidos	36	1	0 (0)	0 (0)	0 (0)	0 (0)
Poza Churince	40	1	0 (0)	0 (0)	0 (0)	0 (0)
Puente Dos Cuatas	60	2	0.3731 (0.2708)	0.0331 (0.0226)	0.0661 (0.1381)	0.0004 (0.0009)
Puente Orosco	39	1	0 (0)	0 (0)	0 (0)	0 (0)
Rio Garabatal	29	1	0 (0)	0 (0)	0 (0)	0 (0)
Tio Candido	22	1	0 (0)	0 (0)	0 (0)	0 (0)

Table 4. Numbers and distribution of CK-A7 alleles in populations of *Cyprinodon atrorus* and *C. bifasciatus*. Alleles cA1-cA4 represent alleles typical of *C. atrorus*, whereas alleles cB1-cB10 represent typical *C. bifasciatus* alleles.

Table 4.
Numbers and Distribution of CK-A7 SSCP Alleles

	CK-A7 Alleles														
Locality	cA1	cA2	cA3	cA4	cB1	cB2	cB3	cB4	cB5	cB6	cB7	cB8	cB9	cB10	Total
C. atrorus populations															
Antiguos Mineros	56														56
Laguna Grande	80														80
Laguna Salada	39	19	1		1										60
Las Playitas	57	2		1											60
Los Gatos	57	6		1											64
MJC 20	41	17													58
San Marcos	20														20
C. bifasciatus populations															
Becerra	2				62	1		4	1	2	2				74
Escobeda					74										74
Juan Santos					74							1	5		80
Los Hundidos					60										60
MJC 26					59									1	60
Mojarral Este					70										70
Mojarral Oeste	1	1			68										70
Poza Churince					78										78
Puente Dos Cuatas	2				116										118
Puente Orosco		2			76										78
Rio Garabatal					47	4	1	8							60
Tio Candido					60										60
	355	47	1	2	845	5	1	12	1	2	2	1	5	1	1280

Table 5. Numbers and distribution of RAG-1 alleles in populations of *Cyprinodon atrorus* and *C. bifasciatus*. Allele rA1 represents the typical *C. atrorus* allele and alleles rB1-rB3 denote the common *C. bifasciatus* alleles.

Table 5.
Numbers and Distribution of RAG-1 SSCP Alleles

	RAG-1 Alleles				
Locality	rA1	rB1	rB2	rB3	Total
C. atrorus populations					
Antiguos Mineros	58				58
Laguna Grande	79	1			80
Laguna Salada	58	2			60
Las Playitas	60				60
Los Gatos	64				64
MJC 20	60				60
San Marcos	19	1			20
C. bifasciatus populations					
Becerra		60	2		62
Escobeda		78			78
Juan Santos		25		55	80
Los Hundidos		60			60
MJC 26		60			60
Mojarral Este		72			72
Mojarral Oeste		70			70
Poza Churince		78			78
Puente Dos Cuatas	2	116			118
Puente Orosco		74			74
Rio Garabatal		57	3		60
Tio Candido		60			60
	400	814	5	55	1274

Table 6. Numbers and distribution of TPI-B4 alleles in populations of *Cyprinodon atrorus* and *C. bifasciatus*. Allele tA1 represents the typical *C. atrorus* allele and alleles tB1-tB2 represent alleles typical of *C. bifasciatus*.

Table 6.
Numbers and Distribution of TPI-B4 SSCP Alleles

	TPI-B4 Alleles			
Locality	tA1	tB1	tB2	Total
C. atrorus populations				
Antiguos Mineros	58			58
Laguna Grande	78	2		80
Laguna Salada	59	1		60
Las Playitas	60			60
Los Gatos	64			64
MJC 20	59	1		60
San Marcos	20			20
C. bifasciatus populations				
Becerra		70	2	72
Escobeda		76		76
Juan Santos		76		76
Los Hundidos		60		60
MJC 26	1	55		56
Mojarral Este		60		60
Mojarral Oeste		72		72
Poza Churince		80		80
Puente Dos Cuatas	2	118		120
Puente Orosco		78		78
Rio Garabatal		58		58
Tio Candido		44		44
	401	851	2	1254

Table 7. P-values for tests of Hardy-Weinberg equilibrium (HWE) in CK-A7, RAG-1, and TPI-B4. Numbers in bold represent significant deviations from HWE after Bonferroni correction.

Table 7.
P-Values for Hardy Weinberg Equilibrium in CK-A7, RAG-1, and TPI-B4.

	CK-A7			RAG-1			TPI-B4		
Locality	Obs Het.	Exp. Het	HWE P-Value	Obs Het.	Exp. Het	HWE P-Value	Obs Het.	Exp. Het	HWE P-Value
C. atrorus populations									
Antiguos Mineros	0	0	-	0	0	-	0	0	-
Laguna Grande	0	0	-	0	0	-	0	0	-
Laguna Salada	0.4000	0.4848	0.3849	0.0667	0.0655	1.0000	0	0	-
Las Playitas	0.1000	0.0977	1.0000	0	0	-	0	0	-
Los Gatos	0.1875	0.2009	0.1120	0	0	-	0	0	-
MJC 20	0.3333	0.4588	0.3776	0	0	-	0	0	-
San Marcos	0	0	-	0.1000	0.1000	1.0000	0	0	-
C. bifasciatus popualtions									
Becerra	0.2432	0.2966	**0.0030**	0.0645	0.0635	1.0000	0.0556	0.0548	1.0000
Escobeda	0	0	-	0	0	-	0	0	-
Juan Santos	0.1500	0.1421	1.0000	0.4250	0.4617	1.0000	0	0	-
Los Hundidos	0	0	-	0	0	-	0	0	-
MJC 26	0.0333	0.0333	1.0000	0	0	-	0	0	-
Mojarral Este	0	0	-	0	0	-	0	0	-
Mojarral Oeste	0	0	-	0	0	-	0	0	-
Poza Churince	0	0	-	0	0	-	0	0	-
Puente Dos Cuatas	0.0168	0.0174	1.0000	0.0168	0.0167	1.0000	0	0	-
Puente Orosco	0.0513	0.0506	1.0000	0	0	-	0	0	-
Rio Garabatal	0.4333	0.3701	1.0000	0.0333	0.0966	0.0514	0	0	-
Tio Candido	0	0	-	0	0	-	0	0	-

Table 8. P-values for tests of linkage disequilibrium between CK-A7, RAG-1, and TPI-B4. Numbers in bold represent significant deviations from linkage disequilibrium after Bonferroni correction.

Table 8. Linkage disequilibrium among loci in *Cyprinodon atrorus* and *C. bifasciatus*

Locality	CK-RAG	CK-TPI	RAG-TPI
C. atrorus populations	-	-	-
San Marcos	-	-	-
Laguna Grande	-	-	0.82142
Laguna Salada	0.07418	0.03228	**0.01420**
Las Playitas	-	-	-
MJC 20	-	0.11769	0.11769
Los Gatos	-	-	-
Antiguos Mineros	-	-	-
C. bifasciatus populations	-	-	-
Poza Churince	-	-	-
Becerra	0.06818	0.99253	**0.00144**
Rio Garabatal	0.68035	0.68035	0.68035
Juan Santos	0.68245	0.85387	0.57675
Mojarral Oeste	0.04913	0.04913	0.04913
Mojarral Este	-	-	-
Puente Orosco	**0.00068**	0.90129	**0.00088**
Puente Dos Cuatas	**0.00145**	**0.00015**	**0.00015**
MJC 26	-	0.91597	0.91597
Escobeda	-	-	-
Escobedita	-	-	-
Tio Candido	-	-	-
Los Hundidos	-	-	-

Table 9. Numbers and distribution of cyt *b* SSCP haplotypes in populations of *Cyprinodon atrorus* and *C. bifasciatus*.

Table 9.
Numbers and Distribution of cyt*b* SSCP Haplotypes

	Mitochondrial Haplotype																				
Locality	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	Total
C. atrorus populations																						
Antiguos Mineros		9															21					30
Laguna Grande	40																					40
Laguna Salada		30																				30
Las Playitas		29	1																			30
Los Gatos		29		1	5	1						1	3				1			1		42
San Marcos	10																					10
Poza Churince	40																					40
C. bifasciatus populations																						
Becerra	40																					40
Escobedita												30										30
Escobeda							29				2	7							1			39
Juan Santos									40													40
Los Hundidos							30															30
MJC 26	1	15					14															30
MJC 20		30																				30
Mojarral Oeste	1						4			8				15	5			3				36
Mojarral Este	8	1					7			5				13	2							36
Puente Orosco	6	14			1		4	3				10				2						40
Puente Dos Cuatas	8	7			6							7				2						30
Rio Garabatal	29																				1	30
Tio Candido							30															30
	183	164	1	1	12	1	118	3	40	13	2	55	3	28	7	4	22	3	1	1	1	663

Table 10. Standard measures of genetic variation in cyt*b* for each population (as labeled in Figure 1).

Table 10.
Standard Measures of Genetic Variation for Each Population at Cyt *b*

Locality	N	Number of Haplotypes	Theta	Gene Diversity	Mean Number of Pairwise Differences	Nucleotide Diversity
C. atrorus populations						
Antiguos Mineros	30	2	0.7573 (0.4762)	0.4345 (0.0699)	1.3035 (0.8379)	0.0041 (0.0029)
Laguna Grande	30	1	0(0)	0(0)	0(0)	0(0)
Laguna Salada	30	1	0(0)	0(0)	0(0)	0(0)
Las Playitas	30	2	0.2524 (0.2524)	0.0667 (0.0613)	0.0667 (0.1416)	0.0002 (0.0005)
Los Gatos	42	8	2.0916 (0.8939)	0.5134 (0.0892)	0.8583 (0.6186)	0.0027 (0.0022)
MJC 20	30	1	0(0)	0(0)	0(0)	0(0)
San Marcos	10	1	0(0)	0(0)	0(0)	0(0)
C. bifasciatus populations						
Becerra	40	1	0(0)	0(0)	0(0)	0(0)
Escobeda	39	4	1.1826 (0.6104)	0.4224 (0.0860)	0.9501 (0.6644)	0.0030 (0.0023)
Escobedita	30	1	0(0)	0(0)	0(0)	0(0)
Juan Santos	40	1	0(0)	0(0)	0(0)	0(0)
MJC 26	30	3	1.0097 (0.5710)	0.5494 (0.0381)	0.7149 (0.5515)	0.0022 (0.0019)
Mojarral Este	36	6	1.4469 (0.7063)	0.7810 (0.0375)	1.7429 (1.0373)	0.0055 (0.0036)
Mojarral Oeste	36	6	1.6881 (0.7855)	0.7587 (0.0487)	1.7810 (1.0547)	0.0056 (0.0037)
Los Hundidos	30	1	0(0)	0(0)	0(0)	0(0)
Poza Churince	40	1	0(0)	0(0)	0(0)	0(0)
Puente Dos Cuatas	30	5	1.2621 (0.6612)	0.8023 (0.0264)	1.7770 (1.0579)	0.0056 (0.0037)
Puente Orosco	40	7	1.6457 (0.7600)	0.7936 (0.0377)	1.7013 (1.0159)	0.0053 (0.0035)
Rio Garabatal	30	2	0.2524 (0.2524)	0.0667(0.0613)	0.0667 (0.1416)	0.0002 (0.0005)
Tio Candido	30	1	0(0)	0(0)	0(0)	0(0)

APPENDIX I

CK-A7 ALLELES

Appendix I- CK-A7 Alleles

cA1

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTTCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

cA2

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTTCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCGTTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

cA3

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTTCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCGTTCACTCTCTTCCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

cA4

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTTCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTCCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

cB1

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG
GAGCAGGTGCAGCTGGTCGTCGACGG

cB2

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

cB3

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGA

ACTGGGACTTATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCA

GTGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGG

TGGAGCAGGTGCAGCTGGTCGTCGACGG

cB4

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA

CTGGGACTTATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG

GAGCAGGTGCAGCTGGTCGTCGACGG

cB5

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGACCTGAA

CTGGGACTTATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG

GAGCAGGTGCAGCTGGTCGTCNACGG

cB6

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGACCTGAA

CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGG

TGGAGCAGGTGCAGCTGGTCGTCGACGG

cB7

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA

CTGGGACTCGTTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG

GAGCAGGTGCAGCTGGTCGTCGACGG

cB8

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACTGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA

CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG

GAGCAGGTGCAGCTGGTCGTCGACGG

cB9

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA

CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTTGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG

GAGCAGGTGCAGCTGGTCGTCGACGG

cB10

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG

GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA

CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG

TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCTGAGGTG

GAGCAGGTGCAGCTGGTCGTCGACGG

APPENDIX II

RAG-1 ALLELES

Appendix II- RAG-1 Alleles

rA1

GTTTCAGCACGTCTTCCAGCTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC

CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCGCTGGCCCAGGC

TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGCGCAAGGGTCTTGT

GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAAGGTAGAGGAGAGC

AGGTCAGCAAAACGTTGTGAGTTAAAACTGTAGCGGCACAGCTGGTCAGGGC

ACTCCTTGGCT

rB1

GTTTCAGCACGTCTTCCAGTTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC

CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCGCTGGCCCAAGC

TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGTGCAAGGGTCTTGT

GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAAGGTAGAGGAGAGC

AGGTCAGCAAAACGTTGTGARTTAAAACTGTAGCGGCACAGCTGGTCAGGGC

ACTCCTTGGCT

rB2

GTTTCAGCACGTCTTCCAGTTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC

CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCGCTGGCCCAAGC

TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGTGCAAGGGTCTTGT

GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAATGTAGAGGAGAGC

AGGTCAGCAAAACGTTGTGAGTTAAAACTGTAGCGGCACAGCTGGTCAGGGC

ACTCCTTGGCT

rB3

GTTTCAGCACGTCTTCCAGTTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC
CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCACTGGCCCAAGC
TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGTGCAAGGGTCTTGT
GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAAGGTAGAGGAGAGC
AGGTCAGCAAAACGTTGTGAGTTAAAACTGTAGCGGCACAGCTGGTCAGGGC
ACTCCTTGGCT

APPENDIX III

TPI-B4 ALLELES

Appendix III- TPI-B4 Alleles

tA1

TGAGAGGGAGGGAGGCATCACGGAGAAGGTGGTGTTTGCTCAGACCAAAGT

CATTGCAGGTATGCAGCTTTCAGCAGGGAAGCTGAGTGATGCAACTCCAATT

CGGCTTTGGGGGTGAAAATAAATCTGCTTGTACAATACCAAGTCTTATCTCAT

TGATAGACAATGTAAAG

tB1

TGAGAGGGAGGGAGGCATCACGGAGAAGGTGGTGTTTGCTCAGACCAAAGT

CATCGCAGGTATGCAGCTTTCAGCAGGGAAGCTGAGTGATGCAACTCCATTT

CGGCTTTGGGGGTGAAAATAAATCTGCTTGTACAATACCAAGTCTTATCTCAT

TGATAGACAATGTAAAG

tB2

TGAGAGGGAGGGAGGCATCACGGAGAAGGTGGTGTTTGCTCAGACCAAAGT
CATCGCAGGTATGCAGCTTTCAGCAGGGAAGCTGAGTGATGCAGCTCCATTT
CGGCTTTGGGGGTGAAAATAAATCTGCTTGTACAATACCAAGTCTTATCTCAT
TGATAGACAATGTAAAG

APPENDIX IV

CYT B HAPLOTYPES

Appendix IV-Cyt *b* Haplotypes

Haplotype *C.* "aguanaval"

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGACTATTTCTGGCAAT

ACACTATACATCAGATATTTCGACTGCCTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGCCGAGGCCTTTATTATGGTTC

CTACCTATACAAAGAAACCTGAAATGTTGGAGTAGTCCTCCTCCTTCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGAGGGCAAATGTCATTTTGA

GGTGCCACAGTAATTACCAACCTCCTGTCCGCTGTCCCTTACGTCGGAGACGC

CCTCGTACAATGAATTTGAGGCGGATTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTTCCCTTCGTAGTAGCTGCCGCTACCATGG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGACTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TGGGATTCGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCC

AACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGC

CCCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGG

TCTATTCCGAATAAGCTAGGAGGAGTTCTCGCTCTTCTAGCATCCATCCTCGT

CCTCATAGTTGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCC

GCCCCTTCACACAATTCCTGTTCTGGCTCTTAGTTGCCGATGTAATGATCCTA

ACATGAATCGGGGGCATACCAGTAGAACACCCCTTCGTCATTATTGGACAAG

TAGCCTCCTTCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCT

TGACTAGAA-ACAAAGTCCTCGGATGATAA

Haplotype A

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGCCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGCGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATAG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAAGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCGCTAGTAACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCAATTCTTCATACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

ATGAATCGGGGGCATACCAGTAGAACACCCCTTCGTCATTATTGGACAAGTA

GCCTCCTTCCTTTACTTCTTCTTATTCCTTGTTCTTTCTCCCACCGCAGCTTG

ACTAGAAAACAAAGTCCTCGGATGATAA

Haplotype B

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAACTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATGG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTCCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

ATGAATCGGGGGCATACCAGTAGAGCACCCCTTCGTCATTATTGGACAAGTA

GCCTCCTTCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCTTG

ACTAGAA-ACAAAGTCCTCGGATGATAA

Haplotype C

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATAG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCA-

ACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTACATGAATCG

GGGGCATACCAGTAGAGCACCCCTTCGTCATTATTGGACAAGTAGCCTCCT

TCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCTTGACTAGAAA

ACAAAGTCCTCGGATGATAA

Haplotype D

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATTACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATGG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCGATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

ATGAATCGGGGGCATACCAGTAGAGCACCCCTTCGTCATTATTGGACAAGT

AGCCTCCTTCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCTTGA

CTAGAAAACAAAGTCCTCGGATGATAA

Haplotype E

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CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGAGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATGG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

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TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACACC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCATC

CTCATAGTCGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

ATGAATCGGGGGCATACCAGTAGAGCACCCCTTCGTCATTATTGGACAAG

TAGCCTCCTTCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCTTG

ACTAGAAAACAAAGTCCTCGGATGATAA

Haplotype F

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TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCGTAGTAGCTGCCGCTACCATGG

TACACCTAGTGTTCCTCCACGAAACTGGGTCCAACAACCCAACAGGGCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

ATGAATTGGGGGCATACCAGTAGAGCACCCCTTCGTCATTATTGGACAAG

TAGCCTCCTTCCTTTACTTCTTCTTGTTCCTTGTTCTTTCTCCCACCGCAGCTTG

ACTAGAAAACAAAGTCCTCGGATGATAA

Haplotype G

ATGGCTAACCTACGAAAAACTCACCCCCTCCTAAAAATTGCAAATAACGCAC

TGGTGGACCTCCCAGCACCAGTAAACATCTCCGCTTGATGAAACTTTGGCTCC

CTCCTGGGACTATGTCTAATTGCCCAGATCTTAACAGGTCTATTTCTGGCGAT

ACACTATACATCAGATATTTCGACTGCTTTCTCATCCGTTGCTCACATCTGCC

GTGACGTTAATTACGGCTGACTCATCCGAAACATGCATGCTAATGGAGCATC

TTTCTTCTTCATCTGTATTTACCTGCATATCGGTCGAGGCCTTTATTATGGCTC

CTACCTATACAAAGAAACCTGAAATGTGGGAGTAATCCTCCTCCTCCTTGTTA

TGATGACAGCCTTTGTTGGGTACGTTTTACCCTGGGGACAAATGTCATTTTGA

GGTGCCACAGTAATCACCAACCTCCTATCCGCTGTCCCTTATGTCGGAGACAC

CCTCGTACAATGAATTTGAGGTGGTTTCTCAGTTGACAACGCAACTTTAACCC

GATTCTTTGCCTTCCACTTCCTCCTCCCCTTCATAGTAGCTGCCGCTACCATGG

TACACCTAGTATTCCTCCACGAAACTGGGTCCAACAACCCAACAGGTCTAAA

TTCAGACGCTGATAAAATCTCCTTCCACCCCTATTTCTCGTATAAGGACCTAC

TAGGATTTGCCCTTCTTCTAGCCACCCTAATTGCACTTGCACTATTCTCGCCTA

ACCTTCTAGGGGACCCAGAAAACTTCACACCCGCCAACCCACTAGTGACGCC

CCCTCACATTAAACCTGAGTGATATTTCCTATTTGCATACGCAATCTTACGGT

CTATTCCCAATAAGCTAGGAGGAGTTCTTGCTCTTCTAGCATCCATCCTCGTC

CTCATAGTCGTGCCAATTCTTCACACATCTAAACAACGAAGCCTAACTTTCCG

CCCCTTCACACAATTCCTGTTCTGGCTCCTAGTTGCTGATGTGATGATCCTTAC

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Haplotype H

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Haplotype I

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Haplotype J

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Haplotype L

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Haplotype M

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Haplotype N

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Haplotype O

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Haplotype P

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Haplotype S

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Haplotype T

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Haplotype U

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APPENDIX V

INDIVIDUALS BY GENOTYPE

Appendix V- Individuals by Genotype

Locality	ID	Cyt *b*	CK	RAG	TPI
Antiguos Mineros	EC0170.01	Q	AA	AA	AA
Antiguos Mineros	EC0170.02	Q	AA	AA	AA
Antiguos Mineros	EC0170.03	Q	??	AA	AA
Antiguos Mineros	EC0170.04	Q	AA	AA	AA
Antiguos Mineros	EC0170.05	Q	AA	AA	AA
Antiguos Mineros	EC0170.06	Q	AA	AA	??
Antiguos Mineros	EC0170.07	Q	AA	AA	AA
Antiguos Mineros	EC0170.08	Q	AA	AA	AA
Antiguos Mineros	EC0170.09	Q	AA	AA	AA
Antiguos Mineros	EC0170.10	Q	AA	AA	AA
Antiguos Mineros	EC0170.11	B	AA	AA	AA
Antiguos Mineros	EC0170.12	Q	AA	AA	AA
Antiguos Mineros	EC0170.13	Q	AA	AA	AA
Antiguos Mineros	EC0170.14	Q	AA	AA	AA
Antiguos Mineros	EC0170.15	B	AA	AA	AA
Antiguos Mineros	EC0170.16	Q	AA	AA	AA
Antiguos Mineros	EC0170.17	B	AA	AA	AA
Antiguos Mineros	EC0170.18	Q	AA	AA	AA
Antiguos Mineros	EC0170.19	Q	AA	??	AA
Antiguos Mineros	EC0170.20	B	AA	AA	AA

Antiguos Mineros	EC0170.21	Q	AA	AA	AA
Antiguos Mineros	EC0170.22	B	AA	AA	AA
Antiguos Mineros	EC0170.23	Q	AA	AA	AA
Antiguos Mineros	EC0170.24	B	AA	AA	AA
Antiguos Mineros	EC0170.25	B	AA	AA	AA
Antiguos Mineros	EC0170.26	B	AA	AA	AA
Antiguos Mineros	EC0170.27	B	??	AA	AA
Antiguos Mineros	EC0170.28	Q	AA	AA	AA
Antiguos Mineros	EC0170.29	Q	AA	AA	AA
Antiguos Mineros	EC0170.30	Q	AA	AA	AA
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Becerra	EC0130.02	A	BB	BB	BB
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Becerra	EC0130.12	A	BB4	BB2	BB2
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Becerra	EC0130.22	A	BB	??	BB
Becerra	EC0130.23	A	BB4	??	BB
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Becerra	EC0130.29	A	BB	??	??
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Becerra	EC9917.10	A	BB	BB	BB
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Escobeda	EC0050.07	G	BB	BB	BB
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Escobeda	EC0050.18	L	BB	BB	BB
Escobeda	EC0050.19	G	BB	BB	BB

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Escobeda	EC9921.08	G	BB	BB	BB
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Escobedita	EC0230.01	L	N/A	N/A	N/A
Escobedita	EC0230.02	L	N/A	N/A	N/A
Escobedita	EC0230.03	L	N/A	N/A	N/A

Escobedita	EC0230.04	L	N/A	N/A	N/A
Escobedita	EC0230.05	L	N/A	N/A	N/A
Escobedita	EC0230.06	L	N/A	N/A	N/A
Escobedita	EC0230.07	L	N/A	N/A	N/A
Escobedita	EC0230.08	L	N/A	N/A	N/A
Escobedita	EC0230.09	L	N/A	N/A	N/A
Escobedita	EC0230.10	L	N/A	N/A	N/A
Escobedita	EC0230.11	L	N/A	N/A	N/A
Escobedita	EC0230.12	L	N/A	N/A	N/A
Escobedita	EC0230.13	L	N/A	N/A	N/A
Escobedita	EC0230.14	L	N/A	N/A	N/A
Escobedita	EC0230.15	L	N/A	N/A	N/A
Escobedita	EC0230.16	L	N/A	N/A	N/A
Escobedita	EC0230.17	L	N/A	N/A	N/A
Escobedita	EC0230.18	L	N/A	N/A	N/A
Escobedita	EC0230.19	L	N/A	N/A	N/A
Escobedita	EC0230.20	L	N/A	N/A	N/A
Escobedita	EC0230.21	L	N/A	N/A	N/A
Escobedita	EC0230.22	L	N/A	N/A	N/A
Escobedita	EC0230.23	L	N/A	N/A	N/A
Escobedita	EC0230.24	L	N/A	N/A	N/A
Escobedita	EC0230.25	L	N/A	N/A	N/A
Escobedita	EC0230.26	L	N/A	N/A	N/A

Escobedita	EC0230.27	L	N/A	N/A	N/A
Escobedita	EC0230.28	L	N/A	N/A	N/A
Escobedita	EC0230.29	L	N/A	N/A	N/A
Escobedita	EC0230.30	L	N/A	N/A	N/A
Juan Santos	EC0087.01	I	BB	B3B3	BB
Juan Santos	EC0087.02	I	BB	BB	BB
Juan Santos	EC0087.03	I	BB9	BB3	??
Juan Santos	EC0087.04	I	BB	B3B3	BB
Juan Santos	EC0087.05	I	BB9	BB	BB
Juan Santos	EC0087.06	I	BB	BB3	BB
Juan Santos	EC0087.07	I	BB	BB3	BB
Juan Santos	EC0087.08	I	BB	BB3	BB
Juan Santos	EC0087.09	I	BB	B3B3	BB
Juan Santos	EC0087.10	I	BB	BB3	BB
Juan Santos	EC0087.11	I	BB	BB3	BB
Juan Santos	EC0087.12	I	BB9	B3B3	BB
Juan Santos	EC0087.13	I	BB	BB3	BB
Juan Santos	EC0087.14	I	BB	BB3	??
Juan Santos	EC0087.15	I	BB	B3B3	BB
Juan Santos	EC0087.16	I	BB	BB	BB
Juan Santos	EC0087.17	I	BB	B3B3	BB
Juan Santos	EC0087.18	I	BB9	B3B3	BB
Juan Santos	EC0087.19	I	BB	BB3	BB

Juan Santos	EC0087.20	I	BB	BB3	BB
Juan Santos	EC0087.21	I	BB	B3B3	BB
Juan Santos	EC0087.22	I	BB	BB3	BB
Juan Santos	EC0087.23	I	BB	B3B3	BB
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Juan Santos	EC0087.25	I	BB	B3B3	BB
Juan Santos	EC0087.26	I	BB	B3B3	BB
Juan Santos	EC0087.27	I	BB9	B3B3	BB
Juan Santos	EC0087.28	I	BB8	B3B3	BB
Juan Santos	EC0087.29	I	BB	B3B3	BB
Juan Santos	EC0087.30	I	BB	B3B3	BB
Juan Santos	EC9915.01	I	BB	BB3	BB
Juan Santos	EC9915.02	I	BB	BB3	BB
Juan Santos	EC9915.03	I	BB	BB3	BB
Juan Santos	EC9915.04	I	BB	B3B3	BB
Juan Santos	EC9915.05	I	BB	B3B3	BB
Juan Santos	EC9915.06	I	BB	BB3	BB
Juan Santos	EC9915.07	I	BB	B3B3	BB
Juan Santos	EC9915.08	I	BB	B3B3	BB
Juan Santos	EC9915.09	I	BB	BB	BB
Juan Santos	EC9915.10	I	BB	BB3	BB
Laguna Grande	EC0317.01	A	AA	AA	AA
Laguna Grande	EC0317.02	A	AA	AA	AA

Laguna Grande	EC0317.03	A	AA	AB	AA
Laguna Grande	EC0317.04	A	AA	AA	AA
Laguna Grande	EC0317.05	A	AA	AA	AA
Laguna Grande	EC0317.06	A	AA	AA	AB
Laguna Grande	EC0317.07	A	AA	AA	AA
Laguna Grande	EC0317.08	A	AA	AA	AA
Laguna Grande	EC0317.09	A	AA	AA	AA
Laguna Grande	EC0317.10	A	AA	AA	AA
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Laguna Grande	EC0317.18	A	AA	AA	AA
Laguna Grande	EC0317.19	A	AA	AA	AA
Laguna Grande	EC0317.20	A	AA	AA	AB
Laguna Grande	EC0317.21	A	AA	AA	AA
Laguna Grande	EC0317.22	A	AA	AA	AA
Laguna Grande	EC0317.23	A	AA	AA	AA
Laguna Grande	EC0317.24	A	AA	AA	AA
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Laguna Grande	EC0317.26	A	AA	AA	AA
Laguna Grande	EC0317.27	A	AA	AA	AA
Laguna Grande	EC0317.28	A	AA	AA	AA
Laguna Grande	EC0317.29	A	AA	AA	AA
Laguna Grande	EC0317.30	A	AA	AA	AA
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Laguna Grande	EC9904.10	A	AA	AA	AA
Laguna Salada	EC0132.01	B	A2A2	AA	AA
Laguna Salada	EC0132.02	B	AA	AA	AA
Laguna Salada	EC0132.03	B	AA	AA	AA
Laguna Salada	EC0132.04	B	AA	AA	AA
Laguna Salada	EC0132.05	B	AA2	AA	AA
Laguna Salada	EC0132.06	B	AA2	AA	AA
Laguna Salada	EC0132.07	B	AA2	AA	AA
Laguna Salada	EC0132.08	B	AA2	AA	AA

Laguna Salada	EC0132.09	B	A2A2	AA	AA
Laguna Salada	EC0132.10	B	AA	AA	AA
Laguna Salada	EC0132.11	B	AA2	AA	AA
Laguna Salada	EC0132.12	B	A2A2	AA	AA
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Laguna Salada	EC0132.18	B	AA	AA	AA
Laguna Salada	EC0132.19	B	AA	AA	AA
Laguna Salada	EC0132.20	B	AA2	AA	AA
Laguna Salada	EC0132.21	B	A2A2	AA	AA
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Laguna Salada	EC0132.27	B	AA2	AA	AA
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Las Playitas	EC9911.01	B	AA	AA	AA

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Las Playitas	EC9911.09	B	AA	AA	AA
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Las Playitas	EC0072.03	B	AA2	AA	AA
Las Playitas	EC0072.04	B	AA	AA	AA

Las Playitas	EC0072.05	B	AA	AA	AA
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Las Playitas	EC0072.07	B	AA	AA	AA
Las Playitas	EC0072.08	B	AA	AA	AA
Las Playitas	EC0072.09	B	AA	AA	AA
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Los Gatos	EC9912.01	D	AA	AA	AA
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Los Gatos	EC9912.03	B	AA	AA	AA
Los Gatos	EC9912.04	B	AA	AA	AA
Los Gatos	EC9912.05	F	AA	AA	AA
Los Gatos	EC9912.06	E	AA	AA	AA
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Los Gatos	EC9912.10	M	AA	AA	AA
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Los Gatos	EC0017.02	B	AA2	AA	AA
Los Gatos	EC0017.03	B	AA2	AA	AA
Los Gatos	EC0017.04	L	AA	AA	AA
Los Gatos	EC0017.05	B	AA2	AA	AA
Los Gatos	EC0017.06	Q	AA	AA	AA
Los Gatos	EC0017.07	B	AA	AA	AA

Los Gatos	EC0017.08	B	AA	AA	AA
Los Gatos	EC0017.11	B	AA	AA	AA
Los Gatos	EC0017.12	B	AA	AA	AA
Los Gatos	EC0017.13	M	AA	AA	AA
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Los Gatos	EC0017.15	B	AA	AA	AA
Los Gatos	EC0017.16	E	AA	AA	AA
Los Gatos	EC0017.17	B	AA2	AA	AA
Los Gatos	EC0017.18	E	AA2	AA	AA
Los Gatos	EC0017.19	B	A2A4	AA	AA
Los Gatos	EC0017.20	B	AA	AA	AA
Los Gatos	EC0017.25	B	AA	AA	AA
Los Gatos	EC0017.26	B	AA	AA	AA
Los Gatos	EC0017.27	B	N/A	N/A	N/A
Los Gatos	EC0017.28	B	N/A	N/A	N/A
Los Gatos	EC0017.29	B	N/A	N/A	N/A
Los Gatos	EC0017.30	B	N/A	N/A	N/A
Los Gatos	EC0018.01	B	AA	AA	AA
Los Gatos	EC0018.02	T	AA	AA	AA
Los Gatos	EC0018.03	B	N/A	N/A	AA
Los Gatos	EC0018.04	B	N/A	N/A	AA
Los Gatos	EC0018.05	B	N/A	N/A	AA
Los Gatos	EC0018.11	M			AA

Los Gatos	EC0018.13	B			
Los Gatos	EC0018.14	E			
Los Hundidos	CLH.01	G	BB	BB	BB
Los Hundidos	CLH.02	G	BB	BB	BB
Los Hundidos	CLH.03	G	BB	BB	BB
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Los Hundidos	EC0043.10	G	BB	BB	BB
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Los Hundidos	EC9913.02	G	BB	BB	BB
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Los Hundidos	EC9913.05	G	BB	BB	BB
Los Hundidos	EC9913.06	G	BB	BB	BB
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MJC20	EC0154.02	B	AA2	AA	AA
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MJC20	EC0154.14	B	AA	AA	AA

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MJC20	EC0154.17	B	AA	AA	AA
MJC20	EC0154.18	B	AA	AA	AA
MJC20	EC0154.19	B	AA2	AA	AA
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MJC20	EC0154.21	B	AA	AA	AA
MJC20	EC0154.22	B	A2A2	AA	AA
MJC20	EC0154.23	B	AA2	AA	AA
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MJC26	EC0126.18	G	BB	BB	BB
MJC26	EC0126.19	A	BB	BB	BB
MJC26	EC0126.20	B	BB	BB	??
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Mojarral Este	EC0088.08	G	BB	BB	??
Mojarral Este	EC0088.09	N	BB	BB	??
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Mojarral Este	EC0088.23	N	BB	BB	BB

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Puente Orosco	EC0074.12	B	BB	BB	BB

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Puente Orosco	EC0074.26	L	BB	BB	BB
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Puente Orosco	EC9914.06	B	BB	BB	BB
Puente Orosco	EC9914.07	B	BB	BB	BB
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Puente Orosco	EC9914.09	L	BB	BB	BB
Puente Orosco	EC9914.10	A	BB	BB	BB
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Rio Garabatal	EC0052.06	A	BB4	BB	BB
Rio Garabatal	EC0052.07	A	BB	BB	BB
Rio Garabatal	EC0052.08	A	BB	BB	BB
Rio Garabatal	EC0052.09	A	BB	BB	BB
Rio Garabatal	EC0052.10	A	BB	BB	BB
Rio Garabatal	EC0052.11	A	BB	BB	BB
Rio Garabatal	EC0052.12	A	BB4	BB	BB
Rio Garabatal	EC0052.13	A	BB4	BB	BB
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Rio Garabatal	EC0052.16	A	BB	B2B2	BB
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Rio Garabatal	EC0052.18	A	BB4	BB	BB

Rio Garabatal	EC0052.19	A	BB	BB	BB
Rio Garabatal	EC0052.20	A	BB	BB	BB
Rio Garabatal	EC0052.21	A	BB4	BB	BB
Rio Garabatal	EC0052.22	A	BB	BB	BB
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Rio Garabatal	EC0052.24	A	BB4	BB	BB
Rio Garabatal	EC0052.25	A	BB	BB	BB
Rio Garabatal	EC0052.26	A	BB2	BB	BB
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San Marcos	EC9922.06	A	AA	AA	AA
San Marcos	EC9922.07	A	AA	AA	AA
San Marcos	EC9922.08	A	AA	AA	AA
San Marcos	EC9922.09	A	AA	AA	AA
San Marcos	EC9922.10	A	AA	AA	AA
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Tio Candido	EC0051.23	G	BB	BB	??
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Tio Candido	EC0051.25	G	BB	BB	??
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Tio Candido	EC9920.04	G	BB	BB	BB

CHAPTER 3

Importance and complexity of exogenous selection in an animal hybrid zone: insights from reciprocal transplant experiments

Abstract:

A key element to understanding hybridization in plants and animals is to determine the relative influences of endogenous and exogenous selection on hybrid zone structure. To date such studies have been explored in many plant systems, but extremely limited in animals. To help remedy this imbalance, we conducted reciprocal transplant experiments with two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus,* to assess the importance of environmental factors in the hybridization dynamics of these species. Results from our experiments corroborate the hypothesis that *C. atrorus* is euryplastic and *C. bifasciatus* stenoplastic, and therefore provide evidence that differential environmental tolerance may be important in regulating the distribution of genetic variation in hybrid zones between these species. However, these differences do not explain why *C. atrorus* is absent from *C. bifasciatus* habitats. This absence is likely determined at least in part by exogenous selection, such as disproportionate predator susceptibility and/or inferior competitive ability of *C. atrorus*. We propose that different exogenous selection pressures, operating in different directions, are likely to be important in regulating hybridization between these species. Although the relative role of endogenous selection in this system remains unknown, this study provides insight into the complexity of exogenous selection in animal hybrid zones.

Introduction

Environmental and genetic variation is often strongly correlated in hybrid zones, with frequency distributions of species-specific markers grading across environmental transitions that separate parental taxa (Barton and Hewitt 1985; Harrison 1986; Rand and Harrison 1989; Cruzan and Arnold 1993; Szymura 1993; Johnson et al. 2001). Similar correlations are also known to occur within species (Powers and Place 1978; Hilbish 1985; Schmidt and Rand 1999, 2001; Dufresne et al. 2002). In intra-specific situations, these patterns are usually thought to result from selection acting near or on marker loci (e.g., *Semibalanus balanoides,* acorn barnacles: Schmidt and Rand 1999, 2001; Rand et al. 2001, 2002), and the selective mechanisms that likely generate such allelic gradients have been experimentally identified in some systems (e.g., *Mytilus edulis,* marine mussels: Hilbish and Koehn 1985; *Fundulus heteroclitus,* killifish: Powers and Schulte 1998). In many hybrid zones, however, the adaptive significance of similar patterns remains uncertain. Historically, hybrids have generally been viewed as genetically inferior, especially in animals, and therefore not meaningfully affected by exogenous selection; however, recent investigations of plant and animal hybrid zones have prompted reconsideration of this viewpoint (reviewed in Arnold 1997, Rieseberg 1997).

In an effort to resolve this controversy, several recent studies have used reciprocal transplant experiments to test for adaptive differences between parental species and their hybrids. Many of these studies have demonstrated significant influences of local adaptation on environment-genotype relationships (Emms and Arnold 1997; Wang et al. 1997; Fritsche and Kaltz 2000; Campbell and Waser 2001, Campbell 2004; but see Via et

al. 2000; Bronson et al. 2003), and probable selective mechanisms have been identified in some cases (*Acyrthosiphon,* pea aphids; Via et al. 2000; *Ipomopsis,* flowering plants: Campbell 2004). Information from some of these and other studies was used by Lexer et al. (2003) to investigate the strength of selection in the wild. Based on findings from this study Lexer et al. argued that experimental hybridization could serve as a powerful tool for gaining a more comprehensive understanding of selection in the wild, especially since multiple species are more likely to segregate for adaptive variation than are single taxa. The above studies provide additional evidence that hybridization is evolutionarily important, particularly in plants.

While there has been a wealth of experimental studies designed to test for the influence of exogenous selection in plant hybrid zones, comparable information from animal hybrid zones remains limited to relatively few systems and studies (e.g., *Bombina,* fire-bellied toads: Kruuk and Gilchrist 1997; MacCallum et al. 1998; Kruuk et al. 1999; Vorndran et al. 2002; *Acyrthosiphon,* pea aphids: Via et al. 2000; *Rana,* leopard frogs: Parris 2001; *Poecile,* chickadees: Bronson et al. 2003). This information gap limits our ability to use hybrid zones as laboratories to investigate the mechanistic bases of adaptation, and also hampers efforts to determine whether introgressive hybridization is of broad evolutionary importance (e.g., Arnold 1997; Dowling and Secor 1997). To remedy this problem, additional, field based experimental studies that assess importance of exogenous selection in animal hybrid zones are required before the general suitability of hybrid zones to investigations of adaptation can be determined.

An excellent opportunity to investigate the importance of exogenous selection on environment-genotype associations in animals involves natural hybridization between *Cyprinodon atrorus* and *C. bifasciatus*, two pupfishes endemic to Cuatro Cienegas, Mexico. Based on morphology, Miller (1968) considered these species to be distantly related, with *C. bifasciatus* representing a basal member of the genus. This conclusion was further corroborated in an allozyme phylogeny of *Cyprinodon* by Echelle and Echelle (1998). Yet despite the relatively distant relationship between these species, extensive hybridization has led to complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus* (Echelle et al submitted; Carson and Dowling unpub.). However, introgression at three nuclear loci surveyed to date (CK-A, TPI-B, and RAG-1) is limited to regions of contemporary hybridization, where there are strong correlations between environmental and species-specific nuclear gene variation (Carson and Dowling unpub.). These findings indicate that endogenous and/or exogenous selection may severely limit exchange of nuclear genes between these species. Several other factors are consistent with an important role for exogenous selection in shaping the distributions of these species. *C. atrorus* and *C. bifasciatus* inhabit opposite extremes of a severe physicochemical gradient, are thought to exhibit large differences in environmental tolerance (Miller 1968, Minckley 1969, Arnold 1972), and display disparate morphological (Miller 1968), ecological (Arnold 1972), and behavioral (Miller 1968; Itzkowitz and Minckley 1969; Minckley and Arnold 1969; Minckley 1969; Arnold 1972; Minckley 1984; Ludlow 2000) characteristics.

Cyprinodon atrorus is widespread and occupies pools, marshes, and lagunas that experience extreme environmental fluctuations on daily and seasonal scales. *C. bifasciatus*, in contrast, is found in physicochemically constant thermal springs and reaches of outflow that exhibit minor to moderate physicochemical variation. Temperature tolerances of *C. atrorus* are among the broadest recorded for aquatic vertebrates, with field measurements ranging from 2.0-47.2°C (Deacon and Minckley 1974). Maximum and minimum temperature tolerances of *C. bifasciatus* are not known, but are thought to be considerably narrower. These species are sympatric in environmentally intermediate regions that connect springheads/thermal outflows to peripheral/terminal habitats. In these areas hybridization is extensive and involves considerable admixture of parental gene pools (advanced backcrosses, etc.).

Correlations between environmental and genetic variation could be generated in part by environmental tolerance differences between *Cyprinodon atrorus* and *C. bifasciatus*; however, existence of such differences has not been experimentally verified. To test whether environmental tolerances of *C. atrorus* and *C. bifasciatus* are significantly different, we conducted reciprocal transplant experiments with these species in summer 2002 and winter 2003. All else being equal, environmental stability of thermal, riverine systems is expected to present minimal physicochemical stress to both species in summer and winter, whereas the environmental severity of peripheral, pool habitats should produce disproportionately higher mortality in *C. bifasciatus* in both summer and winter. Results from this study provide insights into the potential influence

of local adaptation in animal hybrid zones, as well as the selective mechanisms that may maintain adaptive separation of these species despite their extensive genetic connectivity.

Materials and Methods

Experimental sites

Reciprocal transplant experiments with *Cyprinodon atrorus* and *C. bifasciatus* were conducted in summer 2002 (19 July-8 August) and winter 2003 (10-22 January) in the Rio Churince system of the Cuatro Cienegas basin, Mexico. Two experimental sites, representing typical *C. atrorus* and *C. bifasciatus* habitats, were chosen based on previous surveys (Carson unpub.) of genetic and physicochemical variation throughout the Rio Churince system. Habitat for *C. atrorus* was represented by a peripheral side-pool in the upper reaches of the Rio Churince, but typically isolated from the river by a dry channel ~30m long. Area of the side pool varies seasonally from small (~10 m^2, 0.25 m deep) to moderate (>50 m^2, to > 0.75 m deep), depending on evaporation and rainfall. Sediment consists almost entirely of a thick, soft bacterial/algal mat and *Chara*. Surface contact with the Rio Churince is occasionally established following heavy summer rains, but is usually terminated within days to weeks due to the high rates of evaporation during this season. Stagnation at this site is prevented by a combination of shallow habitat, exposure to strong daily winds, and inflow from groundwater percolation/subterranean channels. Habitat for *C. bifasciatus* was represented by a ~10m mid-upper reach of the Rio Churince adjacent to the peripheral side-pool. The Rio Churince habitat consisted of

a stream reach approximately 2m wide by 0.5m deep, with a soft, organically rich sediment composed primarily of snail copropel and decaying vegetation. Water level at this site remains essentially constant year round except for brief, minor increases following heavy rains (summer) or periods of reduced evaporation (winter). Current is steady but not swift and physicochemical variation is low over daily and seasonal bases.

Specimen Collection

Prior to initiation of reciprocal transplant experiments, seines and dipnets were used to collect approximately 60 individuals of each species from the experimental sites. An additional 30 specimens per species were collected at each site/date for characterization of diagnostic molecular markers (see *Genetic Analyses* below). For both species, collections were biased toward juveniles and females due to concerns that the aggressive, territorial nature of mature males would be intensified in the confines of the experimental enclosures, and thus introduce artificially elevated mortality into the experiment. Otherwise, collection was random.

Specific conductance (a proxy measure of salinity) and water temperature were recorded at each collection locality at the time of capture. Individuals were transported to the laboratory (18 km) in separate 45 quart coolers (by species) filled with water from the habitat of the respective species. Additional water was collected from each site for use in acclimation of collected individuals to experimental conditions (see below).

Genetic Analyses

Thirty additional *Cyprinodon* were collected from both the Rio Churince and peripheral side-pool sites at the start of summer and winter experiments. These specimens were preserved in 95% ethanol to provide samples for analysis of species-specific nuclear gene variation of populations at each site. Assessments of the degree and potential seasonality of nuclear gene introgression at both sites were accomplished by analysis of single-stranded conformational polymorphisms (SSCPs) (Glavac and Dean 1993; Takeda et al. 1995; Gerber et al. 2001) at three nuclear gene loci (intron 5 of creatine kinase A [CK-A], part of exon 2 from the recombination activation gene 1 [RAG-1], and intron 4 of triosephosphate isomerase B [TPI-B]); a previous basin wide survey of nuclear gene variation in *Cyprinodon atrorus* and *C. bifasciatus* determined that these species segregate diagnostically different alleles at each locus, with one to five nucleotide differences between species-specific alleles (Carson and Dowling in prep). A representative of each SSCP variant was sequenced using an ABI 377 automated sequencer for confirmation of genotype.

Acclimation to Experimental Conditions

Collections of each species were randomly divided into four 19 L containers per species, with approximately 15 *Cyprinodon atrorus* or *C. bifasciatus* per container. Water temperature in containers was allowed to equilibrate. Acclimation of each group, including controls, to experimental conditions was achieved by serial replacement of water in each container with water from the destined experimental habitat. Replacement

intervals for transplant groups and controls were every hour (summer) or 30 minutes (winter), with 10 percent of the water replaced each time (replacement intervals were interrupted in summer for five hours in the afternoon and 11 hours overnight). Specific conductance was recorded after each replacement and water replacements were terminated after specific conductance was within 200μS of the target value (as determined by specific conductance recorded for each experimental site). The same number of water replacements was conducted for transplant and control groups; however, control "acclimation" entailed replacement of existing water with water of equivalent specific-conductance. Thus, all groups, including controls were treated identically, though specific conductance in control groups did not change during the acclimation phase. For the summer experiment, 17 water replacements were made over 32 hours because no water replacements were made during the night. Four replacements over two hours were made in winter. Fewer water replacements were conducted in winter due to essential equivalence of specific conductance between the Rio Churince and peripheral side-pool. No mortality occurred during acclimation.

Experimental Design

Experimental enclosures were constructed of PVC pipe frames (1m long, 30.5cm diameter) that supported clear Lexan tube inserts. Enclosure ends were covered with nylon mesh with a pore size of 2 mm, which was of sufficient size to maintain flow, but small enough to prevent fish from escaping or entering. A hinged, sealable opening in the top of each enclosure allowed for easy addition and removal of *Cyprinodon*. Side

handles bolted to enclosure ends were used to attach iButton temperature sensors (Maxim Integrated, Dallas Texas), which were set to record water temperature at thirty-minute intervals over the course of each experiment. Cages were numbered 1-16, with eight placed by random order at each site. Mesh ends were faced into the water current in the Rio Churince and in the direction of prevailing winds in the peripheral side-pool.

Prior to introducing *Cyprinodon* to the enclosures, water temperature and specific conductance were measured in the Rio Churince and peripheral side-pool sites. Nineteen-liter containers were next placed and secured in the appropriate habitat until water temperature in the container was equal to that of the surrounding habitat (approximately 30 min). Then, five *Cyprinodon atrorus* or *C. bifasciatus* were randomly chosen and added to each appropriate enclosure. This number was chosen because it represented a reasonable approximation of natural densities of both species, provided adequate sample sizes for statistical analyses, and was small enough to minimize mortality that could result from crowding or aggression in the confinement of the cages. Cage surfaces and ends were cleaned lightly every other day to prevent shading and obstruction of flow through the mesh ends.

Experimental enclosures were checked daily for mortalities and specific conductance was checked every several days. In the summer experiment, dead fish observed during daily inspections were carefully removed from affected tubes to eliminate potential adverse impacts of decaying fish on remaining individuals. To control for handling effects in each case, all tubes (with and without mortalities) were handled similarly. In winter, dead fish were not removed, but were instead only noted,

due to initial concerns that summer mortality of *Cyprinodon bifasciatus* in the peripheral side-pool might have been influenced by combined effects of handling and environmental stress. At the end of the experiments, final specific conductance measures were recorded at each site. All remaining *Cyprinodon* were then removed from the enclosures and mortality/survivorship was recorded for each enclosure at each site. Water temperature variation throughout the experiment was directly downloaded from the iButton temperature sensors onto a computer.

Statistical analyses

Wilcoxon's signed-ranks tests for two groups with paired observations were conducted to determine if there were significant differences in specific conductance between the Rio Churince and peripheral side-pool in summer and winter. This test was also used to determine whether average daily high and low temperatures were significantly different between the two sites in summer and winter. A Monte-Carlo bootstrap simulation in Poptools (Hood 2004) was used to determine if there were significant differences in average temperature between the Rio Churince and peripheral side pool in summer and winter. This test was also used to determine if temperature variance was significantly different between the sites in summer and winter.

Mortality of each species was recorded by counting the number of dead *Cyprinodon* in each enclosure at each site. Association of site, species, survival, and season were tested using log linear analyses (SYSTAT 2000 Version 10.2). Log linear analyses are used to find the most parsimonious (best fit) model for multi-way frequency

tables. These analyses are made through hierarchical removal of factors and interactions from a more saturated model until the data fits the simplest model and χ^2 remains insignificant for the model (SYSTAT 10.2).

Results

Genetic Variation

There was no observed introgression of TPI-B alleles into the *Cyprinodon atrorus* population in the peripheral side-pool in either winter or summer; however, minor introgression of *C. bifasciatus*-specific alleles was observed for CK-A (~1 percent in winter and 3.5 percent in summer) and RAG-1 (~3.5 percent in summer and winter). Winter and summer collections of *Cyprinodon bifasciatus* from the Rio Churince site showed no evidence of introgression at the three loci we surveyed (CK-A, RAG-1, and TPI-B).

Environmental Variation

Wilcoxon's signed-ranks tests for two groups (paired observations) indicated significant differences in specific conductance between Rio Churince and the peripheral side-pool in summer (P=0.012) and winter (P=0.007). The extreme drop in specific conductance in the peripheral side-pool in summer (Figure 1) resulted from monsoon activity the evening before (18 July, 2002) the experiment was initiated. The resultant bi-layer (~10,000µS in the lower layer that surrounded the enclosures vs. 2500µS upper layer) gradually mixed during the experiment until specific conductance equilibrated at a

low of ~3200μS. Monsoon activity also immediately increased specific conductance in the Rio Churince (due to terrestrial salt input) from 2580μS to 4490μS, which gradually declined to pre-July 18 levels of ~2600μS (Figure 1). Specific conductance remained relatively stable throughout the winter experiment at the Rio Churince, ranging from 2595μS to 2641μS. Specific conductance was more variable in the peripheral side-pool, ranging from 2601μS to 3171μS.

Results from the Monte-Carlo bootstrap simulation indicate that average summer temperature (Figure 2) in the peripheral side-pool (26.6 °C; 95 percent C.I.= 26.3-26.8°C) was significantly lower than that in Rio Churince (27.7°C; 95 percent C.I.= 27.6-27.9°C). Differences in temperature variance were highly significant between the peripheral side pool (+/-14.1°C; 95 percent C.I.= 13.1°C to 15.2 °C) and the Rio Churince (4.2°C; 95 percent C.I.= 3.8-4.5 °C). Wilcoxon's rank signs test indicated that average low temperature in the peripheral side pool (21 °C) was significantly lower ($P<0.001$) than in the Rio Churince (23.9 °C). Similarly, average high temperature in the peripheral side pool (35.9°C) was significantly higher ($P<0.001$) than in the Rio Churince (32.6 °C).

Average winter temperature in the peripheral side-pool (12.1°C; 95 percent C.I.=11.8-12.3 °C) was significantly lower than in the Rio Churince (20.9°C; 95 percent C.I.=20.7-21.1 °C) (Figure 2). Temperature variance in the peripheral side pool (+/- 8.3 °C; 95 percent C.I.= 7.3-9.0°C) was significantly higher than in the Rio Churince (5.3 °C; 95 percent C.I.= 4.7-6.1 °C). Wilcoxon's ranked signs test indicated that average low and high temperatures in the peripheral side pool (5.5 °C and 14.3 °C) were significantly lower ($P<0.001$) than comparable measures from Rio Churince (18.5 °C and 25.8 °C).

Mortality

Cyprinodon bifasciatus mortality was low in the Rio Churince for both the summer and winter experiments (Figure 3). This species experienced 10% mortality (2 individuals in the same cage) in summer 2002 and 5% (1 individual) in winter 2003. Two *C. bifasciatus* escaped from one enclosure during placement into the water on the first day of the winter 2003 experiment due to a rupture of the seal between the PVC and mesh end of one cage. Fish were not replaced. The end was reinforced and no mortalities or escapes occurred in this flume thereafter. *C. atrorus* experienced no mortality in the Rio Churince habitat in winter or summer.

In contrast to the Rio Churince, *Cyprinodon bifasciatus* experienced much higher mortality than *C. atrorus* in the peripheral side-pool (Figure 3) in summer and winter. At this site, *C. bifasciatus* mortality was 75% (15 out of 20 individuals) in summer and winter, whereas *C. atrorus* experienced 5% mortality (1 individual) in summer and no mortality in winter at this site. The best log linear model (χ^2= 0.0277, df= 1, P= 0.87) from the multi-way frequency table retained the following variables: site, species, and survival, as well as the interaction terms site x species, site x survival, and species x survival. Thus, survival was dependent on species and site, with *C. bifasciatus* experiencing significantly higher mortality than *C. atrorus* in the peripheral side-pool regardless of season.

Discussion

Comprehensive understanding of processes that generate strong correlations between environmental and genetic variation in animal hybrid zones has been hampered by a lack of experimental studies that evaluate adaptive differences between parental species and/or hybrids. We addressed this problem by conducting reciprocal transplant experiments to assess the role of differences in environmental tolerance in explaining the dynamics of hybridization between two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*. Results from these experiments are consistent with the hypothesis that *C. atrorus* and *C. bifasciatus* are separated by significant differences in environmental tolerance. As predicted for winter and summer experiments, survivorship of both species was high in the physicochemically stable Rio Churince, while the presumably stenoplastic *C. bifasciatus* experienced significantly higher winter and summer mortality than the euryplastic *C. atrorus* in the environmentally severe peripheral side-pool. Nuclear introgression in the study populations was minor ($\leq$3.5% in peripheral side-pool) to undetected (Rio Churince). Therefore, results from these experiments are not likely to have been complicated by inclusion of large numbers of heavily hybridized individuals, thus eliminating the influence of endogenous factors in this result. These findings have significant implications for understanding the influence of exogenous selection on environment-genotype relationships in hybrid zones between these species, and further corroborate growing evidence that exogenous selection can have important influences in animal hybrid zones.

Rio Churince

Although both species exhibited high survivorship in the physicochemically stable Rio Churince, *Cyprinodon bifasciatus* did experience higher mortality than *C. atrorus* at this site in winter and summer. In summer there was a brief increase in specific conductance at this site, which resulted from large inputs of terrestrial salts into the system following a monsoon; however, this did not appear to have adversely affected either species in the Rio Churince enclosures, nor were negative effects observed in natural *C. bifasciatus* populations at this site (i.e., no mortality). In winter, no such salinity increases occurred and only one *C. bifasciatus* mortality was observed. Therefore, limited *C. bifasciatus* mortality at this site was most likely a result of random deaths within the experimental populations, although unmeasured seasonal effects and/or handling stress cannot be ruled out.

Peripheral side-pool

The possibility that experimental artifacts could have influenced the disproportionate mortality of *Cyprinodon bifasciatus* in the peripheral side-pool needs to be carefully considered. For instance, handling during capture, transport, and acclimation could have been more stressful to *C. bifasciatus* than to *C. atrorus*, thus making the former more susceptible to death during the experiment. However, we recorded no *C. bifasciatus* and only one *C. atrorus* mortality out of the approximately 60 individuals per species handled during these stages. Furthermore, because mortality in the *C. bifasciatus*

control (Rio Churince) was low in summer (10% = two individuals) and winter (5% = one individual), it is unlikely that stress related to handling before or during the experiment was responsible for *C. bifasciatus* high mortality in the peripheral side-pool enclosures.

Mortality differences between *Cyprinodon atrorus* and *C. bifasciatus* could also have resulted from insufficient time for *C. bifasciatus* to physiologically adjust from a stable to a severe environment during the acclimation period. However, we believe this, too, is a minor concern. First, *C. bifasciatus* chosen for this experiment were collected from a mid-to-upper stream segment of the Rio Churince system, where temperature variation is moderate, not from the head-spring where the thermal regime is essentially constant. Thus, *C. bifasciatus* collected from this location have experienced at least some degree of temperature variation. Second, care was taken to gradually acclimate *C. bifasciatus* to peripheral side-pool conditions. Although water of lower specific conductance occurred in upper layers of this habitat when the experiment was initiated in summer, specific conductivity in the layer where enclosures were placed remained high for several days (Figure 1). Thus re-exposure of *C. bifasciatus* to water of lower salinity was not likely to have been abrupt. Finally, environmental circumstances may change relatively quickly in this habitat under natural conditions and these are the very types of environmental changes that are likely to be common in areas where *C. atrorus* and *C. bifasciatus* distributions meet.

We conclude that mortality differences between *Cyprinodon atrorus* and *C. bifasciatus* in the peripheral side-pool likely reflect biologically significant phenomena.

However, because physicochemical dynamics in this habitat were dissimilar during winter and summer experiments, factors that influenced mortality may also have differed between these periods. In winter, there were statistically significant differences in specific conductance between the Rio Churince and peripheral side-pool, but these differences were well within a range normally experienced by *C. bifasciatus* in nature (Minckley 1969; Arnold 1972; Carson unpublished). Therefore, winter mortality of *C. bifasciatus* in the peripheral side-pool was more likely related to temperature rather than salinity (specific conductivity) variation. The markedly divergent temperature profiles of these habitats were almost certainly of physiological consequence. While the absolute range (low to high) of winter temperature variation was similar between the Rio Churince and peripheral side-pool (12.0°C vs. 13.5°C, respectively), maximum temperature in the side-pool (19.0°C) only approached the mean temperature of the Rio Churince (~20.9°C). Similarly, minimum (5.5°C) temperature in the peripheral side-pool was not only significantly below that of Rio Churince (~14.0°C), but well below any temperature previously recorded for *C. bifasciatus* habitats (~14-15°C) from throughout the basin (Carson unpublished). Extreme winter mortality of *C. bifasciatus* in the peripheral side-pool supports speculation by Minckley (1969) that this species is highly sensitive to low temperatures.

Causes of summer mortality in *Cyprinodon bifasciatus* are likely to have been more complex than those in winter. In summer, variation in both temperature and salinity (as indicated by changes in specific conductivity) was high. The absolute range (low to high) of temperature variation in the peripheral side-pool (~15.0°C) was nearly 60%

higher than that observed in the Rio Churince (9.5°C), whereas maximum temperature (36.0°C) was greater than any known to occur in natural *C. bifasciatus* habitats (~33-34°C in summer). Therefore, the level and magnitude of temperature variation at this site may have contributed to the high mortality of *C. bifasciatus* at this site.

Salinity change was also dramatic in summer, as monsoon rains created a salinity bi-layer that gradually mixed to diminish specific conductance from approximately 10,000µS to 3200µS in the peripheral side-pool. Salinity spiked briefly in the Rio Churince (~4500µS) as well, but soon returned to normal levels (~2500µS). Thus, moderate, but short lived increases in specific conductance did not have obvious negative effects on *Cyprinodon bifasciatus* in Rio Churince; however, the significantly greater salinity changes in the peripheral side-pool may have had important influences on the high level of *C. bifasciatus* mortality observed at this site.

Variation in both temperature and salinity in the peripheral side-pool in summer, as well as the potential influence of important, but non-measured factors, preclude determination of which variables might have had the most influence on mortality of *Cyprinodon bifasciatus*. It is also possible that interaction between temperature and salinity had important influences on mortality in *C. bifasciatus* in both the summer and winter experiments. This study was not designed to address this latter possibility, but such interactions are known to influence mortality in fishes. In a study of the importance of salinity and temperature on growth, food intake, and food conversion, Kinne (1960) noted that mortality of *Cyprinodon macularius* was highest in low salinity/high

temperature situations. [0] This pattern is at least consistent with observed *C. bifasciatus* mortality in the peripheral side-pool in summer. However, a more tightly controlled experimental approach would be required to determine the relationships among temperature, salinity, and mortality in *C. bifasciatus,* and how those relationships differ in magnitude from *C. atrorus*.

Implications for relationships between environmental and genetic variation

Significant mortality differences between *Cyprinodon atrorus* and *C. bifasciatus* in the peripheral side-pool suggest that differences in physiological tolerance influence the distribution of genetic variation across the environmental gradients that separate these species. Although the limited scope of this experiment make it impossible for us to establish the relative importance of seasonal influences, patterns do emerge that can help explain the distributions of *C. bifasciatus*-specific alleles in this system. For example, results from the winter experiment indicate that *C. bifasciatus* is highly sensitive to low water temperatures whereas the summer experiment provides experimental evidence that *C. bifasciatus* is also poorly adapted to large and/or relatively rapid fluctuations in temperature and/or salinity. Because riverine habitats maintain similar salinities throughout the year (~2500-2800μS) it is unlikely that salinity (alone) is as important as temperature in determining the riverine distribution of *C. bifasciatus*. These conclusions are supported by observations (Minckley 1969; Carson, pers. obs.) that *C. bifasciatus* distribution expands downstream in summer as riverine habitats acquire more stable,

thermal spring-like temperature profiles, but contracts in winter as downstream reaches become colder.

Extreme salinity and temperature variation exert especially strong selection against *Cyprinodon bifasciatus* in more peripheral habitats. The preponderance of these environments is in the middle to lower reaches of the Rio Churince, thus *C. bifasciatus* is most likely to come in contact with these areas in summer, as *C. bifasciatus* range expands downstream during this season. The extreme physicochemical conditions that prevail at these sites are likely to inhibit *C. bifasciatus* from entering except when environmental conditions ameliorate (e.g., following monsoons). However, the dynamic nature of these habitats ensures that *C. bifasciatus* occasionally become stranded (Carson, personal observation), at which point selection on these individuals is likely to be intense because summer evaporation rates produce rapid increases in environmental severity at these sites. Furthermore, *C. bifasciatus* that remain trapped until winter are then likely to suffer intense selection associated with low temperatures.

Cyprinodon atrorus and *C. bifasciatus* hybridize extensively in the middle to lower reaches of the Rio Churince, and along interfaces of peripheral/riverine habitats throughout this system. Hybridization between these species may thus expose adaptive variation for environmental tolerance to local selection along the physicochemical gradients that separate *C. atrorus* and *C. bifasciatus*. Although we have not experimentally tested whether there are environmental tolerance differences among hybrid classes, spatial patterns of genetic variation in this system are consistent with this possibility (Carson unpubl.), as hybridized populations predominately occur in

physiologically intermediate habitats. Furthermore, preliminary data on temporal changes in genetic variation across the Rio Churince hybrid zone indicate that there are local, seasonal shifts in the frequency of species-specific alleles in riverine and peripheral habitats (Carson unpubl.). These frequency changes are consistent with environmentally-dependent pulses of selection against *C. bifasciatus*-like hybrids in this system. However, environmental severity is likely to vary in any given season due to the stochastic nature of desert ecosystems; therefore, there is also expected to be inter-annual variability in the magnitude of seasonal shifts in species-specific allele frequencies.

Significant mortality differences between *Cyprinodon atrorus* and *C. bifasciatus* in the peripheral side-pool experiments suggest that differences in environmental tolerance may have significant influences on the distribution of genetic variation across environmental gradients that separate these species. These results indicate that narrow environmental tolerance of *C. bifasciatus* probably has a significant influence on the restriction of this species' distribution to physicochemically stable habitats. Consequently, stenoplasticity of this species may also be important in limiting introgression of *C. bifasciatus* genes into *C. atrorus* populations.

Observed environmental tolerance differences, however, fail to explain the absence of *Cyprinodon atrorus* (and *C. atrorus*-specific alleles) from the most environmentally stable habitats in this system (i.e., areas where *C. bifasciatus* is most abundant). Physicochemical conditions in *C. bifasciatus* habitats are clearly within the survival and reproductive ranges of *C. atrorus*, given experimental results from this study and the diversity of habitats in which this species naturally occurs (Miller 1968, Minckley

1969, Carson, pers. observ.). Similarly, geographic barriers between these species are transient to absent, as *C. atrorus* populations are distributed in a mosaic pattern throughout the Rio Churince system and are commonly in close proximity to and have direct connection with stable habitats. Since movement of *C. atrorus* into *C. bifasciatus* habitats is not limited by physical barriers, other constraints must prevent or inhibit *C. atrorus* from occupying habitats characteristic of *C. bifasciatus*.

Two factors that may limit *Cyprinodon atrorus* occurrence in stable habitats are predation vulnerability and competitive exclusion. While little is known about competitive interactions in this system, evidence from *Cyprinodon rubrofluviatilis* (Echelle et al. 1972), a pupfish ecologically similar to *C. atrorus*, suggests that the more generalist *C. atrorus* may not be adapted to compete against the highly specialized fish fauna with which *C. bifasciatus* is associated. And although the predation hypothesis has not been experimentally tested, considerable observational evidence suggests that predation probably has strong negative influences on *C. atrorus*. For example, several studies have demonstrated that most *Cyprinodon*, of which *C. atrorus* is characteristic, are poorly equipped to survive in the presence of piscivorous fishes (Minckley et al. 1991). Most members of the genus inhabit severe environments that are largely devoid of such predators. In addition, at least one species of *Cyprinodon, C. arcuatus,* is known to have gone extinct due to introduction of a predatory fish species (*Micropterus salmoides*) into its native spring (Minckley et al. 1991), whereas other species have experienced dramatic population reductions (Contreras-B. 1978; Miller and Pister 1971; Pister 2001; Minckley 1991 et al.) or local extirpations following introduction of

predatory fishes (Williams and Deacon 1986; Minckley et al. 1991). *Cyprinodon bifasciatus*, in contrast, is a notable exception within the genus. It lives in very stable aquatic environments, coexists with a complex fish fauna that includes several highly predatory species, such as *Micropterus* and *Cichlasoma,* and exhibits behaviors consistent with highly evolved predator avoidance (Arnold 1972; Minckley 1969). Furthermore, the frequency distribution of *C. bifasciatus*-specific alleles in the Rio Churince system closely parallels the distribution of most other fish species throughout this system (compare to Minckley 1984; Carson pers. obs.), with presence and abundance of non-cyprinodontoid fishes declining with increased habitat variability. In contrast, *C. atrorus*-specific allele frequency shows a strong negative correlation with increased environmental stability and species richness/abundance. Thus *C. atrorus* and *C. bifasciatus* are likely to exhibit significant differences in their ability to successfully co-exist with predatory fishes or compete with complex fish faunas, Such selective factors may in turn may be important elements limiting the distribution of *C. atrorus* and *C. atrorus*-like hybrids. Study of predator susceptibility and competitive differences between these fishes is critical to gaining a comprehensive understanding of distributional and hybridization dynamics between these species.

Together, these patterns suggest that alternate adaptive differences between *Cyprinodon atrorus* and *C. bifasciatus* (e.g., environmental vs. predation tolerance, competitive inferiority vs. superiority) are likely to govern the distributional limits of these species. Life history characteristics that make *C. bifasciatus* well adapted to environmentally benign, predator-rich habitats may limit its ability to persist in severe

and fluctuating environments. For example, Brown and Lomolino (1998) note that species adapted to favorable environments may not be able to meet the energy requirements of harsh systems due to an evolutionary predisposition to allocate proportionately more resources to reproduction and biotic interactions. Similarly, life history traits that make *C. atrorus* well suited to extreme environments may impose a negative cost of adaptation with respect to its ability to survive in favorable habitats. For example, *C. atrorus* is a generalist and thus may not be able to compete against the numerous, highly specialized fishes that occur in *C. bifasciatus* habitats (Echelle et al. 1972). Furthermore, *C. atrorus'* lack of evolution alongside predatory fishes may make this species ill-equipped to respond to predatory fishes. Such costs of adaptation are known to be important in regulating the distributions, and turnover, of species across aquatic habitat gradients (Wellborn et al. 1996). However, the genetic underpinnings of disparate adaptive traits in *C. atrorus* and *C. bifasciatus* segregate in regions of hybridization and, therefore, may be exposed to varied local selection across the environmental gradients that divide these taxa. As such, exogenous selective influences, which operate in different directions and are possibly seasonally variable, are likely to have important influences on the regulation and dynamics of hybridization between these species. Further experimental studies are required to determine the significance of local adaptation in hybrids, as well as the relative roles of exogenous and endogenous selection in this system.

Conclusions

Results from this study demonstrate that *Cyprinodon atrorus* and *C. bifasciatus* differ significantly in environmental tolerance, which may in turn have important influences on the dynamics of hybridization between these species. However, these differences do not fully explain the partitioning of genetic variation across the environmental gradients that separate these taxa. Alternative selection pressures, such as predation and competition in species-rich assemblages, are likely to limit the distribution of *C. atrorus* and thus may also be important in regulation of hybridization between these species. We propose that contrasting exogenous factors that operate in different directions are likely critical to governance of hybridization between *C. atrorus* and *C. bifasciatus*. Although the relative influences of exogenous and endogenous selection remain unknown for this system, our study is significant because it provides insight into the complex means by which adaptive selection may operate in animal hybrid zones. This study also supports the argument that both plant and animal hybrid zones are valuable systems for experimentally addressing the nature and mechanisms of adaptation.

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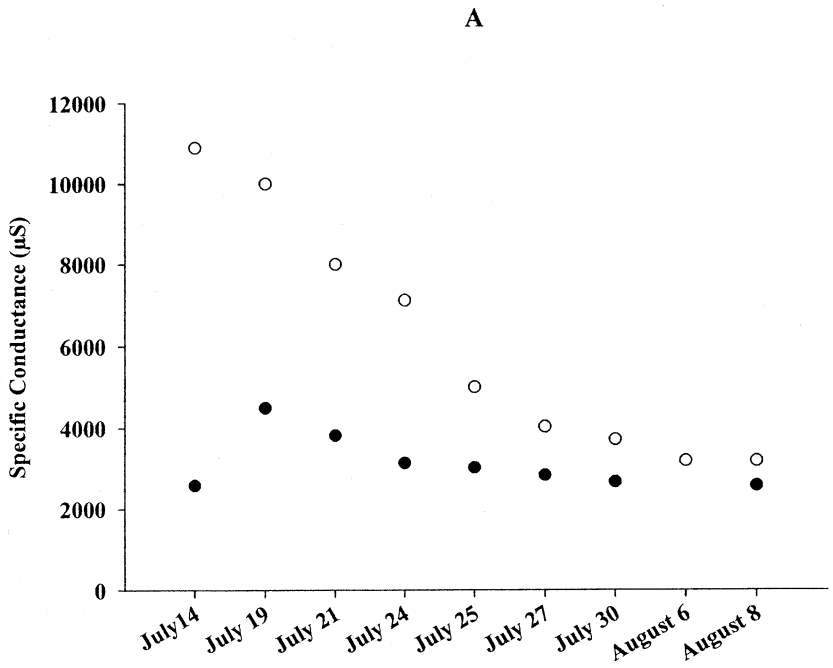
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Figure 1. Variation in specific conductance in the peripheral side-pool and Rio Churince in summer (Panel A) and winter (Panel B). The peripheral side-pool and Rio Churince are represented by open and closed circles, respectively.


A
Specific Conductance (µS)
12000
10000
8000
6000
4000
2000
0
July14
July 19
July 21
July 24
July 25
July 27
July 30
August 6
August 8

B

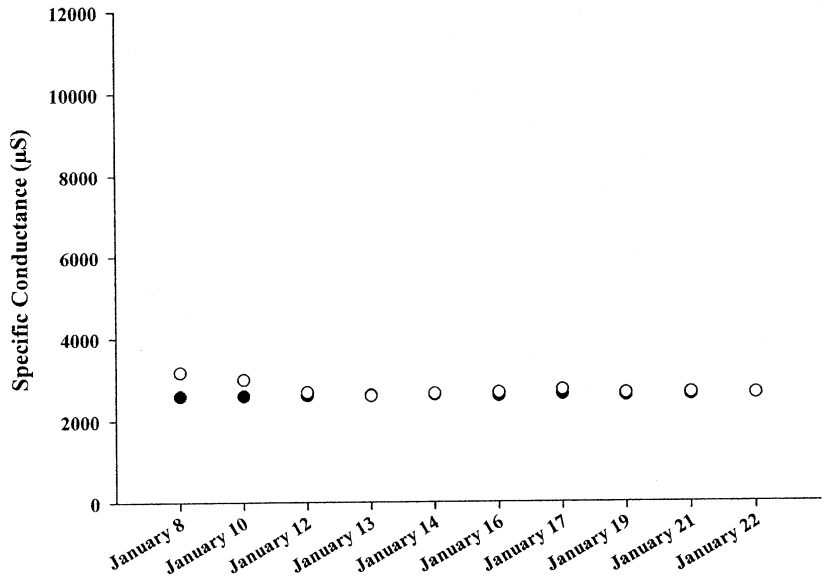
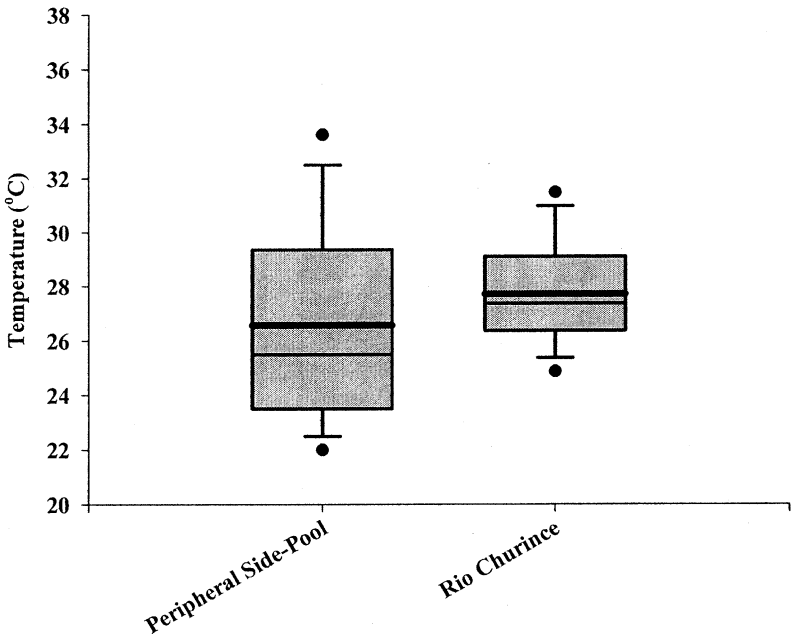

Specific Conductance (µS)
12000
10000
8000
6000
4000
2000
0
January 8
January 10
January 12
January 13
January 14
January 16
January 17
January 19
January 21
January 22

Figure 2. Variation in temperature (°C) in the peripheral side-pool and Rio Churince in Summer (Panel A) and winter (Panel B). Average and median temperatures are respectively indicated by the thick and thin line across each box. The box represents the 25th and 75 percentiles, with bars representing the 5th and 95th percentiles. High and low temperatures at each site are represented by black circles.

A


38
36
34
32
30
28
26
24
22
20
Temperature (°C)
Peripheral Side-Pool
Rio Churince

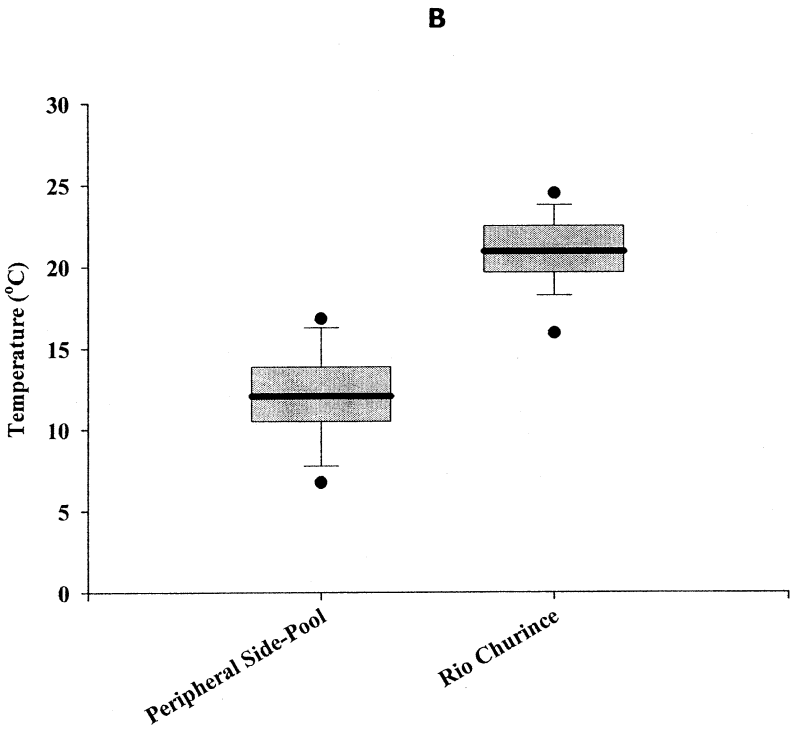
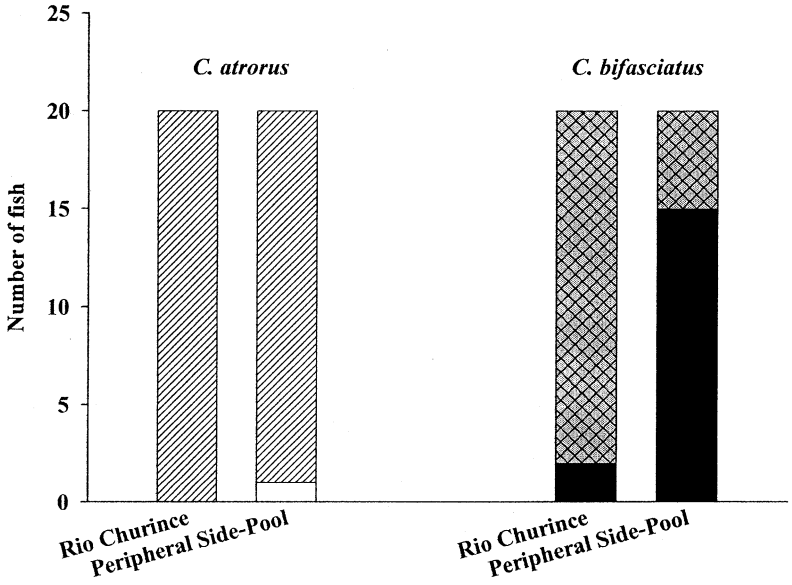
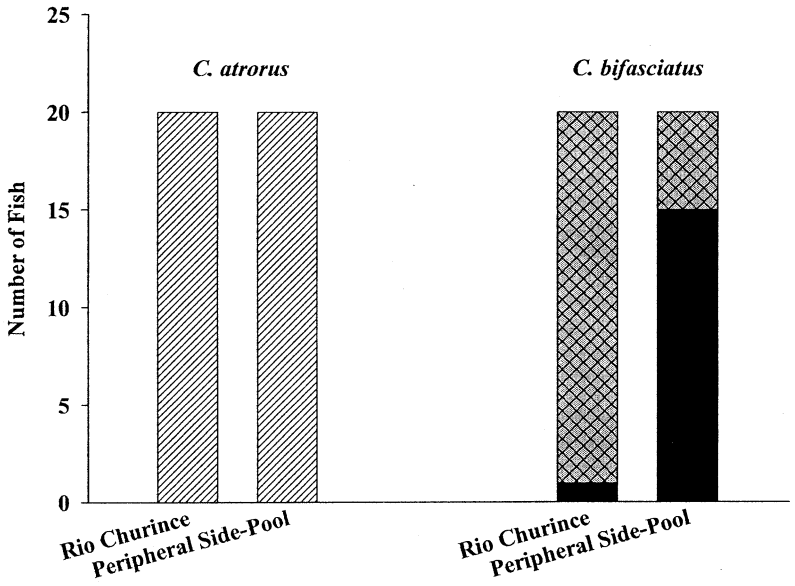

B
30
25
20
15
10
5
0
Temperature (°C)
Peripheral Side-Pool
Rio Churince

Figure 3. Mortality of *Cyprinodon atrorus* and *C. bifasciatus* in the peripheral side-pool and Rio Churince in summer (Panel A) and winter (Panel B). Live and dead *C. atrorus* are represented by cross-hatched bars and white bars, respectively. Live and dead *C. bifasciatus* are represented by diamond-hatched and black bars, respectively.

A


25
20
15
10
5
0
Number of fish
C. atrorus
C. bifasciatus
Rio Churince
Peripheral Side-Pool
Rio Churince
Peripheral Side-Pool

B


25
20
15
10
5
0
Number of Fish
C. atrorus
C. bifasciatus
Rio Churince
Peripheral Side-Pool
Rio Churince
Peripheral Side-Pool

CHAPTER 4

Spatio-temporal relationships between environmental and genetic variation in an animal hybrid zone

Abstract

Hybrid zones have long been studied for the insights these systems can provide about speciation, with considerable attention devoted to understanding environmental and spatial structures, but limited attention to temporal dynamics. In this study, spatio-temporal analyses were conducted to examine the relationship between environmental and genetic variation in a hybrid zone between two pupfish species, the euryplastic *Cyprinodon atrorus* and the stenoplastic *C. bifasciatus*. On a spatial scale, a significant positive correlation was found between *C. atrorus* allele frequency and highly variable environments, while there was also a significant positive correlation between increased *C. bifasciatus* allele frequency and thermally buffered environments, a pattern at least partially explained by the physiological characteristics of these species. On a temporal scale, we found that frequency of diagnostic alleles in the hybrid zone tracked environmental change over time, with *C. bifasciatus* allele frequency declining in cold, hot and variable conditions, but increasing when thermal regimes of habitats acquired a spring-like thermal profile. Conversely, *C. atrorus* allele frequency increased with extremely cold, hot, and variable temperatures. Given the euryplastic nature of *C. atrorus*, it remains unknown why this species does not occupy *C. bifasciatus* habitats; however, it is thought that factors such as predation susceptibility and competitive exclusion may be involved. While the relative importance of endogenous and exogenous

selection in hybrid zones between *C. atrorus* and *C. bifasciatus* is not known, patterns found in this study suggest that environmental variation is critical to governance of hybridization between *C. atrorus* and *C. bifasciatus*. Furthermore, this study demonstrates the importance exogenous selection can have in animal hybrid zones and reveals the critical need to assess temporal, as well as spatial, patterns of environmental and genetic variation within hybrid zones.

Introduction

Hybrid zones have long been studied for insights these systems can provide about the nature of speciation. Given that hybrid zones often lie along ecotones and are exposed to substantial environmental variation, it is imperative that hybrid zone studies address both spatial and temporal aspects of hybridization. However, such studies of animals have generally been confined to temporally static descriptions of hybrid zone pattern and process. This bias has primarily stemmed from a long-standing assumption that hybrid zone dynamics are regulated primarily by endogenous selection (universal inferiority of hybrids) and therefore the additional effort required from temporal studies may not have been justified. Instead, studies of hybrid zones involving animals have focused on the evolution of pre- and post-zygotic barriers to gene exchange (e.g., Harrison 1990). However, several lines of evidence (Harrison 1986, Cruzan and Arnold 1993, Grant and Grant 1993, Arnold 1997, Rieseberg 1997, Emms and Arnold 1997, Kruuk and Gilchrist 1997, MacCallum et al. 1998, Kentner and Mesler 2000, Johnston et al. 2001, Parris 2001, Vines et al. 2003, Fitzpatrick and Shaffer 2004, Gee 2004, Grant et

al. 2004, Chapter 3) suggest that regulation of plant and animal hybrid zones is significantly influenced by environmental (exogenous) selection, introducing the possibility that these systems can exhibit short or long term changes associated with daily, seasonal or longer term environmental change. As such, it has become increasingly important to assess temporal as well as spatial relationships in these dynamic systems.

Several recent studies have incorporated a temporal element into analysis of hybrid zone structure and maintenance (Arntzen and Wallis 1991, Grant and Grant 1993, Gill 1997, Britch et al. 2001, Dasmahapatra et al. 2002, Gilg and Hilbish 2003, Gee 2004, Grant et al. 2004). Results from some of these works have detected hybrid zone movement (Arntzen and Wallis 1991, Gill 1997, Britch et al. 2001, Dasmahapatra et al. 2002), superior fitness of hybrids under certain environmental regimes (Grant and Grant 1993, Grant et al. 2004), and species/hybrid response to environmental change (Grant and Grant 1993, Gee 2004, Grant et al. 2004). While it is likely that temporal change in many hybrid zones occurs over longer periods than covered by typical studies, evidence from Grant and Grant (1993), Gee (2004) and Grant et al. (2004) indicate that consideration of spatio-temporal dynamics in hybrid zones can provide critical insights into the regulatory dynamics of hybrid zones.

Investigation of temporal dynamics of hybrid zones can potentially shed light on the relative importance of endogenous and exogenous selection in these systems. For example, cline movement may be precipitated by either endogenous or exogenous selection (Barton and Hewitt 1985), but the amount of movement expected varies for the

two types of selection. Clines maintained primarily by endogenous selection should come to rest in a density trough that may or may not lie in an ecotone (Barton and Hewitt 1985) and movement may not consistently track environmental change. However, if hybrids respond to environmental variation (seasonal as well as long term), then the position of the hybrid zone should track environmental changes. Thus, movement characteristics of a particular hybrid zone will depend on the relative contributions of both endogenous and exogenous selection to hybrid zone regulation.

The interaction between two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, presents an excellent system to conduct spatio-temporal analyses of the relationship between environmental and genetic variation in hybrid zones. These species are endemic to Cuatro Cienegas, Mexico and have multiple generations per year (Arnold 1972) allowing these species to quickly respond to short-term (e.g., seasonal) changes in environment, which makes them superb subjects for the analysis of patterns of genetic variation across generations and seasons. In addition, these species occupy opposite habitat extremes in a desert spring ecosystem: *C. atrorus* prefers environmentally severe habitats of the basin floor (primarily marshes, playa lakes, riverine edges, and ephemeral pools) whereas *C. bifasciatus* favors physicochemically moderate to constant environments of thermal springs and their environmentally buffered riverine outflows. In habitats where *C. atrorus* occur, temperature and salinity variation can be extreme. Daily temperatures in these habitats can fluctuate as much as 30°C and seasonal temperature changes may be in excess of 45°C (Carson unpubl.). Similarly, salinity in *C. atrorus* habitats can change dramatically over days to weeks, varying between 1500µS and

greater than 16000μS, depending on site and season. In contrast, habitats of *C. bifasciatus* (springs and their most thermally buffered outflows) exhibit low levels of temperature variation, with fluctuations of only a few degrees (3-5 °C) on daily and seasonal bases. Even in the most peripheral *C. bifasciatus* habitats the lowest winter temperatures are generally above 15°C and maximum summer temperatures are near 30-34°C. Specific conductance is also nearly constant, generally ranging from ~2500-3000μS among sites and normally varying by only 200-300μS (Carson unpubl.) within a site.

Reciprocal transplant experiments with *C. atrorus* and *C. bifasciatus* indicate that *C. bifasciatus* suffers high mortality when exposed to *C. atrorus* habitats (Chapter 3), whereas *C. atrorus* exhibits low mortality in *C. bifasciatus* habitats. It is thought that *C. atrorus* is highly susceptible to predation and is prevented from occupying *C. bifasciatus* habitats, at least in part, by the diverse array of predatory fish species that co-occur with *C. bifasciatus*. *Cyprinodon bifasciatus* exhibits morphological and behavioral adaptations (e.g., fusiform body, schooling) that make it less susceptible to predation, whereas *C. atrorus* is a squat-bodied fish and does not exhibit the sophisticated behavioral responses to predation of *C. bifasciatus* (Minckley 1969, Arnold 1972, Minckley pers. comm.). It is also possible that other factors, such as competitive exclusion, prevent *C. atrorus* from occupying *C. bifasciatus* habitats (cf. Echelle et al. 1972).

Despite these differences *C. atrorus* and *C. bifasciatus* hybridize extensively (i.e., dominated by advanced backcrosses) where their ranges overlap in environmentally

intermediate habitats. Given the adaptive differences between *C. atrorus* and *C. bifasciatus*, hybridization between these species is governed, at least in part, by exogenous (environmental) selection. What is unknown is how exogenous selection is manifested over time. The tendency for *C. bifasciatus* to extend its range downstream in summer (Minckley 1969, Minckley pers. comm.) as conditions become more spring-like, and contract in winter as environments become more severe, suggests that the relationship between genetic and environmental variation in hybrid zones between these species may also respond to seasonal environmental change. However, spatio-temporal relationships between environmental and genetic variation have not previously been assessed.

One system of natural hybridization between *C. atrorus* and *C. bifasciatus* occurs in the Rio Churince. This system is relatively small (3.5-4 km long) and thus easily tractable for hybrid zone studies. Within the system there is a strong environmental gradient (salinity, temperature variation, etc.) running from the headspring to a terminal laguna. Upper reaches represent typical habitat for *C. bifasciatus* and are characterized by environmental amenability, with low variation in temperature and salinity. *Cyprinodon atrorus*, in contrast, inhabits the terminal laguna as well as cienega and peripheral habitats; in these habitats environmental variation is severe, with great fluctuations in temperature and salinity on daily to seasonal scales. Hybrids predominate in intervening riverine reaches and peripheral habitats where environmental variation is generally moderate.

To test for an influence of exogenous selection in hybrid zones between *C. atrorus* and *C. bifasciatus*, spatio-temporal sampling was conducted across the Rio Churince system. Genetic variation at three diagnostic nuclear gene loci (creatine kinase A intron 7 [CK-A7], a portion of exon 1 from the recombination activation gene [RAG-1], and triosephosphate isomerase B intron 4 [TPI-B4]) was measured, as were specific conductance (salinity) and daily temperature variation. If seasonal environmental variation influences hybrid zone dynamics in this system, we expected to find the following:

Prediction 1. Downstream riverine reaches should show decrease in *C. bifasciatus* allele frequency between early and late winter (December/January vs. March) as these populations are exposed to cold temperatures, but an increase in *C. bifasciatus* alleles from late winter to summer (March vs. August) as conditions in these reaches become more physicochemically spring-like.

Prediction 2. Fishes from peripheral habitats should exhibit a decrease in *C. bifasciatus* allele frequency between early and late winter (December/January vs. March) as these populations are exposed to cold temperatures, and an additional decrease in *C. bifasciatus* allele frequency from late winter to summer (March vs. August) as these habitats desiccate and in turn experience increased temperature and salinity extremes.

Materials and Methods

Study system

The Rio Churince was selected as a model system for examination of hybridization between *C. atrorus* and *C. bifasciatus*. The system is characterized by a thermal spring source (Poza Churince, *C. bifasciatus* habitat; site CHU 01; Fig. 1) that flows into the Rio Churince and terminates in a large evaporative laguna (Laguna Grande, *C. atrorus* habitat; sites CHU 16 and CHU 17; Fig. 1). Along the transition from spring to evaporative laguna, the Rio Churince takes a winding course in its upper reaches before it empties into a large intermediate laguna (Laguna Intermedia, represented by site CHU 04; Fig. 1). Also flowing into Laguna Intermedia is a marsh system to the northeast (CHU 06 and CHU 07). Laguna Intermedia then empties into a more linear reach of the Rio Churince, which is broken up by a series of peripheral habitats (CHU 10 and CHU 11) and small pools (CHU 13-15; Fig. 1) derived from subsidence and horse traffic. Throughout the system is a series of peripheral pool/marsh habitats (CHU 5, 8, 9, 10 and 12; Fig. 1). The middle and lower reaches consist of a mosaic of habitats.

At each collection site and date, specific conductance was measured using a YSI 85 specific conductance meter. Temperature for each site/collecting trip was also recorded using iButton temperature sensors (Maxim Integrated). iButtons were attached to wooden stakes and placed in each habitat about six inches below the water-line.

Temperature was then recorded every hour for one to three weeks, depending on length of the sampling interval.

Samples

In December 2000 and March 2001, fourteen sites (CHU 1, 2, 4-6, 9-17; Fig. 1) were sampled. Seventeen sites (CHU 1-17; Fig. 1) were sampled in August 2001, January 2002, March 2002, August 2002, January 2003, March 2003, and August 2003. In accordance with permit limitations, 30 individuals were randomly sampled using seine and dip-nets for each site and date. Specimens were immediately preserved in 95% ethanol and DNA was extracted as described in Tibbets and Dowling (1996).

Analysis of Molecular Markers

A survey of nuclear gene [creatine kinase-A intron 7 (CK-A7), recombination activation gene exon 1 (RAG-1), and triosephosphate isomerase B intron 4 (TPI-B4)] variation was previously conducted (Chapter 2) to identify species-specific markers for analysis of the relationship between environmental and genetic variation in the Rio Churince hybrid zone. Variation at these loci was found to be diagnostic for these species and useful for investigation of the relationship between environmental and genetic variation in the Rio Churince hybrid zone. Sequence variation was characterized through analysis of single stranded conformational polymorphisms (SSCPs - Glavac and Dean 1993; Takeda et al. 1995) using α-^{32}P-dATP (Cetus-Perkin Elmer Corp) labeled DNA fragments. For CK-A7, the primers CK-F1 (5'-

CCCAAGTTYGAGGAGATCCTGAC-3') and CK-R1 (5'-CCGTCGACGACCAGCTGCACCTG-3') (Quattro and Jones 1999) were used to amplify a 282 bp fragment using the following PCR conditions: (94°C 30 sec; 60°C 30 sec; 72°C 30 sec; 30 cycles). For RAG-1, the primers Rag-B (5'-TTGGAAGTGTAGAGCCAGTGGT-3') and RAG-Cpup (5'-ATGGCGTGCTACCTGTCC-3') were used to amplify a 355 bp fragment using the following PCR conditions: (94°C 30 sec; 55°C 30 sec; 72°C 30 sec; 30 cycles). For TPI-B4, the primers TPI-B4F2 (5'-GCATYGGGGAGAAGCTRGAT-3') and TPI-B4R1 (5'-AGAACCACYTTRCTCCAGTC-3') (Merritt and Quattro 2001) were used to amplify a 213 bp fragment using the following PCR conditions: (94°C 30 sec; 55°C 30 sec; 72°C 30 sec; 35 cycles). Polymerase chain reactions (PCRs) were run in 5uL total volume per sample. When amplification was completed, four uLs of stop solution (bromophenol blue and xylene cyanol) were added to each PCR product, denatured at 95°C for 3 minutes, and then placed on ice. SSCP gels were then run at room temperature for 18 hours at 6W on six percent (RAG-1) or eight percent (CK-A7 and TPI-B4) acrylamide gels (37.5:1 acrylamide:bis-acrylamide, 1X TBE). Gels were dried and exposed to Kodak XAR film for 12-36 hours. Ambiguous haplotypes or haplotypes that were difficult to score across the gel were re-run on additional gels (or sequenced) until all haplotypes were resolved. Multilocus genotypes for each individual are presented in Appendices I-IX. DNA sequences for diagnostic nuclear gene markers at CK-A7, RAG-1, and TPI-B4 are represented in Appendix X.

Both strands were sequenced from at least one representative of each SSCP variant from each gel using an ABI 377 automated sequencer. The amplification parameters to generate PCR products for sequencing were the same as those used in SSCP except α-^{32}P-dATP was not used and reaction volumes were larger (25 uL). Products were cleaned by centrifugation using Ultrafree MC® filter units (Millipore Corp.) as directed by the supplier. All sequences were aligned by eye in MacDNAsis (Hitachi Corporation 1996).

Statistical analyses

Population genetic parameters (e.g., allele frequencies, heterozygosities, etc.) and tests of single and multilocus equilibrium were generated for each site-by-date combination using Arlequin (Schneider et al. 2000). Multiple linear regression analysis was used to test for correlation between allele frequency (dependent variable; arcsine square root transformed) at each locus and specific conductance and temperature variance (independent variables). Stepwise backward linear regression was run with the threshold for removal of terms set at or above 0.15 and significance level set to 0.05. Spatial and temporal variation in allele frequency was examined using RxC G-tests of independence using the Williams correction as described by Sokal and Rohlf (1995). For each date/site combination in which one or more cells contained a zero (one allele absent), 0.5 was added to each cell. Significance level was set at 0.05 for all tests.

In addition, selection coefficients necessary to maintain cline width were estimated based on reasonable approximation of *Cyprinodon* dispersal distances (Echelle

pers. comm.) following the method of Barton and Gale (1993). Average dispersal estimate of 0.1 km/generation was chosen as a reasonable approximation of dispersal of *C. atrorus* and *C. bifasciatus* (Echelle pers. comm.). Cline width was based on allele frequency across sample sites (CHU01-CHU17), with width spanning from sites with greater than 20% to those with less than 80% frequency of *C. atrorus* alleles.

Results and Discussion

Clines in allele frequency were generally coincident and concordant across the Rio Churince hybrid zone (Figures 2-10). Although all populations were in Hardy-Weinberg equilibrium for all loci (Tables 1-9), within the zone there was considerable linkage disequilibrium between loci (Tables 10-12). At sites where populations were morphologically pure or mildly introgressed there was little to no linkage disequilibrium, whereas linkage disequilibrium was high in heavily introgressed populations (Tables 10-12; Figures 2-10). Hybrids were relatively common, comprising 33% of all individuals, with F_1s representing ~ 15% of hybrid genotypes and 5% of all individuals. Together, these factors suggest that endogenous selection may play an important role in regulation of hybridization of these fishes. In fact, such patterns have been used to identify tension zones, where hybrid zone structure is maintained by a balance between selection against hybrids and dispersal of parental species into the hybrid zone (Barton and Hewitt 1985, Barton and Gale 1993). However, in a compelling re-assessment of a number of classical tension zones, Arnold (1997) demonstrated that coincidence and concordance of clines,

as well as presence of significant linkage disequilibrium, do not negate an important role for exogenous selection in classical tension zones.

Indeed, there is reason to believe that coincidence and concordance of clines, as well as significant linkage disequilibrium and relatively high frequency of F_1 hybrids (15% of hybrids), do not rule out an important role for exogenous selection in the *Cyprinodon* hybrid zone. In particular, habitats in this hybrid zone form a mosaic, with strong local gradients in specific conductance (salinity) and temperature variation (Figures 11 and 12). Along these gradients, there is a significant association between environmental variation and species-specific allele frequency (Tables 14, 15, and 16), with *C. atrorus* alleles predominating in the most severe habitats (Figures 2-10; Tables 1-9) and *C. bifasciatus* alleles characterizing the most stable, environmentally amenable habitats (Figures 2-10; Tables 1-9). Although frequency of *C. atrorus* alleles was not strongly correlated with specific conductance (Tables 14, 15, and 16), there was a significant negative correlation between temperature variation and *C. bifasciatus* allele frequency across the hybrid zone for each of the three nuclear genes (CK-A7, RAG-1, and TPI-B4; Tables 14, 15, and 16). At environmental extremes, there was fixation of *C. bifasciatus* alleles at all three loci in the most environmentally stable habitats and fixation or near fixation of *C. atrorus* alleles at all three loci in the most environmentally variable habitats. In intermediate environments that were more similar to the severe *C. atrorus* habitats there was a predominance of *C. atrorus* and *C. atrorus*-like hybrids (e.g., CHU08, 10, 12; Figures 2-10; Tables 1-9), whereas in intermediate environments more similar to the environmentally buffered *C. bifasciatus* habitats there was a high

occurrence of *C. bifasciatus* and *C. bifasciatus*-like hybrid genotypes (e.g., CHU04; Figures 2-10; Tables 1-9). These patterns are in agreement with the previously demonstrated stenoplastic nature of *C. bifasciatus* (Chapter 3), although they do not explain why *C. atrorus*-like genotypes are absent from *C. bifasciatus* habitats, which may instead be related to predation susceptibility and competitive inferiority of *C. atrorus* (Chapter 3) and/or endogenous selection. The high frequency of advanced backcrosses (~85 % of introgressed genotypes) in both directions (Table 3) also suggests that some hybrid genotypes may have relatively high fitness. Such a pattern has been found in other hybrid zones (Arnold and Hodges 1995), where certain classes of hybrids are as fit or more fit than parental genotypes in at least some habitats. Together, these patterns suggest that environmental selection plays an important role in regulation of hybridization between *C. atrorus* and *C. bifasciatus*.

Assessment of Predictions

If hybridization between these pupfishes has been influenced by environmental variation, we expected to see two phenomena. According to the first prediction, downstream riverine reaches should show a decrease in *C. bifasciatus* allele frequency between early and late winter as these populations are exposed to cold conditions, but an increase in *C. bifasciatus* alleles in summer due to range extension by *C. bifasciatus* / *C. bifasciatus*-like hybrids as more physicochemically spring-like extend downstream. Equivocal support was found for the first prediction. Here, the predicted results were expected because *C. bifasciatus* is known to expand its range downstream in spring and

early summer as riverine physicochemical conditions become more spring-like (Minckley 1969), but contract its range in winter as downstream water temperatures become colder. Of the cases in which there was a significant change (Tables 17-19) in allele frequency between late winter and summer (March vs. August) in downstream riverine sites, seven cases supported and five cases were in disagreement with this prediction (Tables 17-19; Figures 2-10). This may have resulted from having missed the downstream migration of *C. bifasciatus* and *C. bifasciatus*-like hybrids that occurred in spring and early summer. However, human activity may also have been a contributing factor, as modification of the headspring habitat in years two and three led to an abnormal increase in salinity in downstream reaches following input of terrestrial salts after summer monsoons. Such conditions are not thought to be amenable to *C. bifasciatus*. Furthermore, if parental *C. bifasciatus* migrated downstream in early summer, but migrated back upstream in late summer following monsoons (i.e., high salt input), then any juvenile/larvae from spring/early summer crosses between *C. atrorus* and *C. bifasciatus* that remained in these downstream habitats may have escaped capture in August due to their small size. For these reasons our sampling in summer may have been biased towards *C. atrorus*.

There was, however, strong support for the prediction that *C. bifasciatus* allele frequency should decrease in these habitats between early and late winter (December/January vs. March). For example, between December and March 2001, there was a significant decrease in *C. bifasciatus* allele frequency for at least one locus in two riverine (CHU 13 and 14) habitats. A similar pattern was observed in winter 2002, when

there was a significant decrease in *C. bifasciatus* allele frequency between January and March at two riverine (CHU 13 and 14) sites. In 2003, this pattern was observed at three riverine sites (CHU 13, 14, and 15). This general pattern is congruent with reciprocal transplant experiments (Chapter 3), which indicate *C. bifasciatus* to be poorly adapted to cold temperatures.

The second prediction stated that there should have been a decrease in *C. bifasciatus* allele frequency in peripheral habitats between early and late winter (December/January and March) as these populations became exposed to cold conditions and an additional decrease in *C. bifasciatus* allele frequency in summer as these habitats became desiccated and developed increased temperature and salinity extremes (increased variance and upper limits). Evidence consistent with the second prediction was found, where populations in peripheral habitats tended to show a decrease in the frequency of *C. bifasciatus* alleles between early and late winter, as well as an additional decrease in the frequency of *C. bifasciatus* alleles between late winter and summer (August) (Tables 17, 18, and 19; Figures 2-10). For example, between December and March 2001, there was a significant decrease in *C. bifasciatus* allele frequency for at least one locus in peripheral (CHU 09 and 11) habitats. A similar pattern was observed in winter 2002, when there was a significant decrease of *C. bifasciatus* alleles between January and March at four peripheral (CHU05, 08, 09, 10) sites. In 2003, this pattern was observed between January and March at only one peripheral site (CHU 10).

Between late winter (March) and summer (August) 2001 there was a significant decrease in the frequency of *C. bifasciatus* alleles for at least one locus at three peripheral

sites (CHU 05, 10, and 12). Also, between March and August 2002, there was a significant decrease in *C. bifasciatus* allele frequency for at least one locus at five peripheral sites (CHU 05, 08, 09, 10, and 11). In 2003, there were three peripheral sites (CHU 05, 07, 08) that demonstrated a significant decrease in *C. bifasciatus* alleles between March and August. The only reversal of this trend occurred in site CHU 10 where there was a significant increase in *C. bifasciatus* alleles between March and August. Other peripheral sites had high frequencies of *C. atrorus* alleles in March 2003 and thus showed no significant decrease in *C. bifasciatus* allele frequency between March and August 2003. While it is unknown how *C. atrorus*/*C. atrorus*-like and *C. bifasciatus*/*C. bifasciatus*-like individuals interact (e.g., competitive exclusion), the above patterns imply that in winter and summer *C. bifasciatus*-like genotypes are relatively less fit than *C. atrorus*-like genotypes in the environmentally severe peripheral habitats.

Distribution of C. atrorus

While this study indicates that regulation of hybridization between *C. atrorus* and *C. bifasciatus* is strongly influenced by spatio-temporal environmental variation, our results do not explain why *C. atrorus* alleles are absent from *C. bifasciatus* habitats. In fact, results from reciprocal transplant experiments (Chapter 3) clearly demonstrate that *C. atrorus* is capable of living in the physical environmental regimes typical of *C. bifasciatus* habitats. It is likely that other factors, such as predation susceptibility and inter-specific competition, are important in regulating the distribution *C. atrorus* and *C. atrorus*-like hybrids (Chapter 3). To address mechanisms that regulate the distribution of

C. atrorus- and *C. bifasciatus* alleles, future research should focus on parameters that limit the distribution of *C. atrorus*, as well as further investigation into the environmental variables that influence hybridization dynamics in this system.

Spatio-Temporal Variation in Hybrid Zones

It is becoming increasingly clear that to understand the evolutionary consequences of hybridization we must obtain insight into the relative importance of exogenous and endogenous selection in the regulation of hybrid zone dynamics. With respect to exogenous selection, the relationship of spatial patterns of environmental and genetic variation across hybrid zones has been of particular interest. To date, numerous studies have assessed the spatial relationships between environmental and genetic variation in hybrid zones, often demonstrating a strong correlation between environmental parameters and genetic signature. For example, in hybrid zones between the fire-bellied toads *Bombina bombina* and *B. variegata* Barton and colleagues have demonstrated a strong correlation between environmental and genetic variation (MacCallum et al. 1998, Vines et al. 2003). Similarly, studies by Arnold and colleagues have demonstrated that in Louisiana irises there is a strong correlation between environmental and genetic variation (Cruzan and Arnold 1993) and that environmental variation is important in determining hybrid fitness, with some classes of hybrids being as fit or more fit than their parents (Emms and Arnold 1997). These patterns strongly suggest an important role of exogenous selection in shaping the structure of some hybrid zones.

While the above studies demonstrate that spatial environmental variation can be important in regulation of hybrid zones, recent studies suggest that it is also critical to assess temporal dynamics of environmental variation. However, although several studies have focused on spatio-temporal dynamics of hybrid zones (Arntzen and Wallis 1991, Grant and Grant 1993, Gill 1997, Britch et al. 2001, Dasmahapatra et al. 2002, Gilg and Hilbish 2003, Gee 2004, Grant et al. 2004), few have explicitly addressed the role of spatio-temporal environmental variation in the regulation of hybrid zones (Grant and Grant 1993, Gee 2004, Grant et al. 2004). In studies that have included a time component, there has been a clear demonstration that temporal environmental variation can play a significant role in hybridization dynamics. For example, Grant and Grant (1993) and Grant et al. (2004) have demonstrated how hybridization, in concert with El Nino climatic oscillations, has led to directional phenotypic change in Darwin's finches (*Geospiza*). Similarly, Gee (2004) has shown that genetic composition of hybrid quail populations changes with inter-annual climatic (rainfall amount) change.

Selection Coefficients

Regardless of the relative roles of endogenous and exogenous selection in the *Cyprinodon atrorus/C. bifasciatus* system, selection against introgression is strong. Cline width varied seasonally from 0.52 km to 0.82 km for CK-A7, 0.52 km to 0.93 km for RAG-1, and 0.52 km to 0.93 km for TPI-B4 (Tables 20-22). Selection coefficients, based on 0.1 km dispersal for both species, ranged from 0.12 to 0.30 for CK-A7, 0.09 to 0.30 for RAG-1, and 0.09 to 0.83 for TPI-B4 (Tables 20-22). The high selection coefficient

(0.83) for TPI-B4 may be artificially inflated, as an upstream site, CHU08, was not sampled in December 2000 or March 2001, but was sampled in remaining periods and consistently set the upper limit of the cline. These patterns suggest that selection against introgression is strong across this hybrid zone. In fact, the hybrid zone is a mosaic, and our approximations are likely to underestimate the actual intensity of selection because local allele frequency clines can occur over very short distances.

One important observation from this study was that the width of the hybrid zone varied seasonally and inter-annually. The upstream boundary of the cline (CHU08) remained stable across seasons and years, but the position of the lower end was highly variable, ranging from CHU11 to CHU13-CHU15 (Figures 2-10; Tables 20-22). For example, in year one the lower cline limit in December was at CHU 14 for CK-A7, RAG-1, and TPI-B4, but by March had contracted to CHU 13 for CK-A7 and RAG-1, and CHU 11 for TPI-B4. However, by August of that year the lower cline limit had expanded to CHU 14 for CK-A-7 and RAG-1, and CHU15 for TPI-B4. These patterns, and their associated selection coefficients (Tables 20-22), are consistent with cold temperatures having a negative influence of *C. bifasciatus*-like individuals, with cline contraction in winter and expansion in summer.

A more varied picture emerged in year two. The lower end of the cline in January for CK-A7 and RAG-1 was at CHU14, while that of TPI-B4 was positioned at CHU15. By late winter (March), cline width had contracted to CHU13 for CK-A7, expanded to CHU15 for RAG-1, and remained stable at CHU15 for TPI-B4. By summer (August), cline width remained stable at CHU13 for CK-A7, and had contracted to CHU 13 for

both RAG-1 and TPI-B4. Why cline width between early and late winter (January vs. March) only contracted at CK-A7 rather than at all three genes is unknown, but possibly could have resulted from a milder winter, as temperatures were more moderate in the winter of year two. Similarly, failure of the cline to expand in width in summer is puzzling and difficult to explain. However, this divergent pattern relative to year one may be explained in part by human activity at the headspring that resulted in increased salinity in the system in summer following monsoon activity (see above, Carson pers. obs.). This increased salinity may have negatively impacted *C. bifasciatus*-like individuals and prevented further expansion of *C. bifasciatus* alleles in the lower reaches of the system in summer (August).

In year three there was a dramatic contraction in cline width between early and late winter (January vs. March), with CK-A7 and RAG-1 contracting from CHU 14 to CHU 11 and TPI-B4 contracting from CHU 15 to CHU 11. This again is consistent with *C. bifasciatus* being negatively impacted by cold conditions that prevail in winter. However, expected expansion of cline width between late winter and summer (March vs. August) was not observed for any of the three genes, rather cline width remained stable between late winter and summer for all three genes. This pattern, again, is possibly related to human development at the headspring that lead to increased salinity in the lower reaches of the system in summer following monsoon activity. The relationship between cline width and environmental variation was marked, as there was a significant association between environmental variation (temperature variance, but not specific conductance) and allele frequency (Tables 14, 15, and 16). *Cyprinodon atrorus* alleles

were significantly associated with environments that exhibited high temperature variance, whereas *C. bifasciatus* alleles were significantly associated with environments that had low temperature variance (Figures 13, 14, and 15). In environments with moderate to high temmperature variance, hybrids predominated (Figures 13, 14, and 15). Thus, the cyclical pattern of cline width varying with season does provide strong evidence that hybridization between these species is significantly regulated by environmental factors. Specifically, such cyclical changes in cline width are predicted by models that assume an important role for environmental selection, but are not predicted by models that assume a primary role for endogenous selection. However, the relative importance of endogenous and exogenous selection in this system remains unknown.

Conclusions

The present study is unique in that it has laid out explicit predictions for how the distribution of genetic variation in a hybrid zone should change with seasonal variation in climatic conditions. These predictions were largely met. Most importantly, we found that presence of *C. bifasciatus* and *C. bifasciatus*-like genotypes is negatively correlated with extreme environmental variation, especially temperature variation. Sites that exhibited the greatest temperature variation were also the sites with the lowest frequency of *C. bifasciatus* alleles. Conversely, sites with the least temperature variation demonstrated the highest frequency of *C. bifasciatus* alleles. At sites with intermediate to high temperature variation there was a preponderance of hybrid genotypes. Sites with temperature profiles most similar to typical *C. atrorus* habitats exhibited a low frequency

of *C. bifasciatus*-like hybrids, whereas sites with temperature profiles more similar to typical *C. bifasciatus* habitats had a high proportion of *C. bifasciatus*-like genotypes. As environmental patterns changed spatially over different seasons, so too did the distribution of genetic variation within the hybrid zone. Because these spatio-temporal changes were predictable, there is strong evidence that exogenous selection plays a critical role in the governance of hybridization between *C. atrorus* and *C. bifasciatus*.

While the relative roles of endogenous and exogenous selection in this system remain unknown, our results suggest that both types of selection are important in governance of hybridization between *Cyprinodon atrorus* and *C. bifasciatus*. Coincidence and concordance of clines, strong linkage disequilibrium within the hybrid zone, and relatively high frequency of F_1 hybrids suggests that endogenous selection contributes to the regulation of hybridization between *C. atrorus* and *C. bifasciatus*. Conversely, an important role for exogenous selection is supported by predictable change in the distribution of genetic variation with climate change and a high frequency of advanced backcross genotypes that segregate according to habitat.

Overall, findings from this study have two significant implications. First, this study further confirms that animal hybrid zones can be regulated, at least in part, by environmental variation. In this study we demonstrated that not only is inter-annual variation important, but intra-annual, seasonal variation must also be considered. Consequently, this pattern suggests that similar trends may exist in other hybrid systems. Second, this study provides clear evidence that in order to gain a comprehensive understanding of hybrid zone dynamics in animal and plant systems, it can be critical to

address temporal, as well as spatial, patterns of environmental and genetic variation across hybrid zones. Future research in this system should be directed to quantify the relative roles of exogenous and endogenous selection in hybrid zones between *C. atrorus* and *C. bifasciatus*, and particular attention should be paid to factors that limit *C. atrorus* distribution.

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Figure 1. Map of the Rio Churince system, Cuatro Cienegas, Coahuila, Mexico showing location of sample sites. Sites 1- 17 correspond to collection identification numbers CHU01-17.

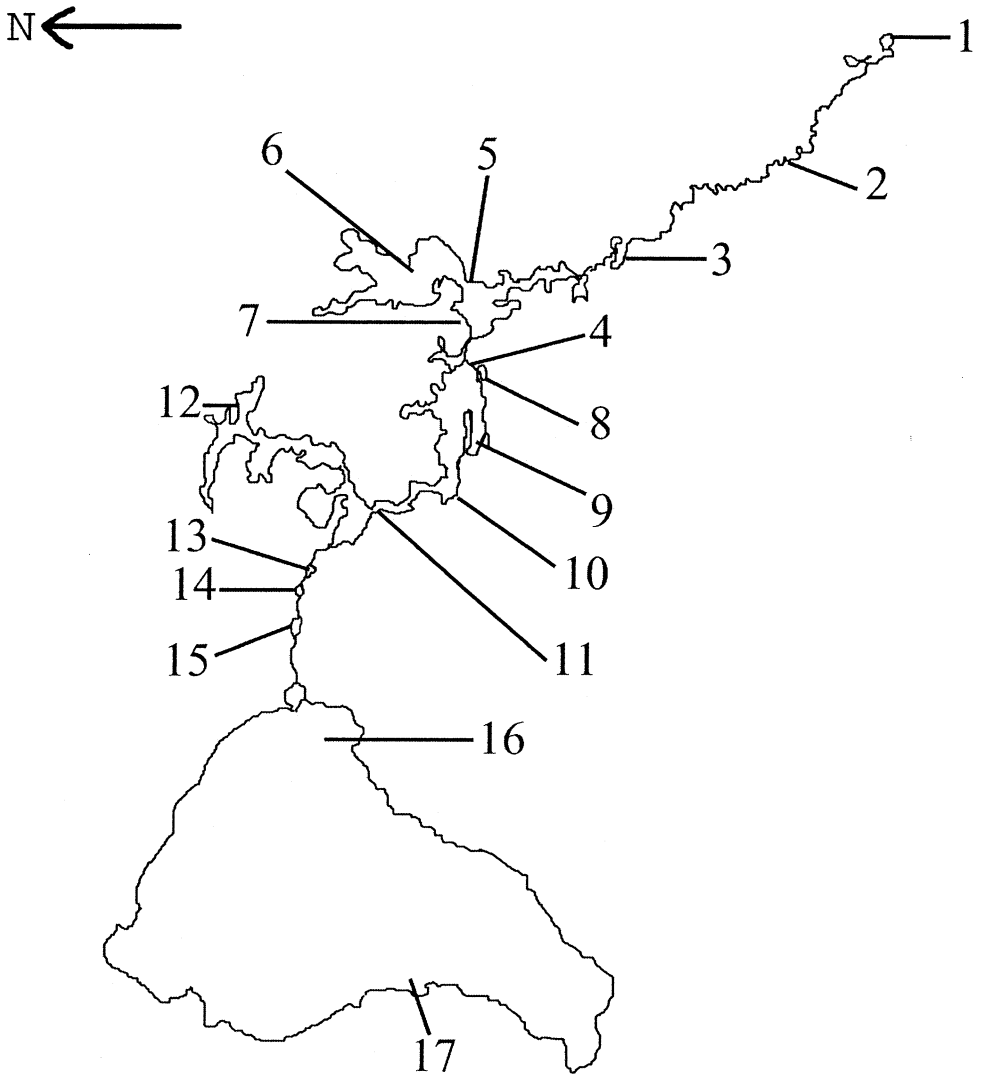

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Figure 2. CK-A7 variation in December 2000, and March and August 2001 across sites CHU 01-17. From left to right pie diagrams represent December, March, and August, respectively. CHU 03, 07, and 08 are represented only by August 2001. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

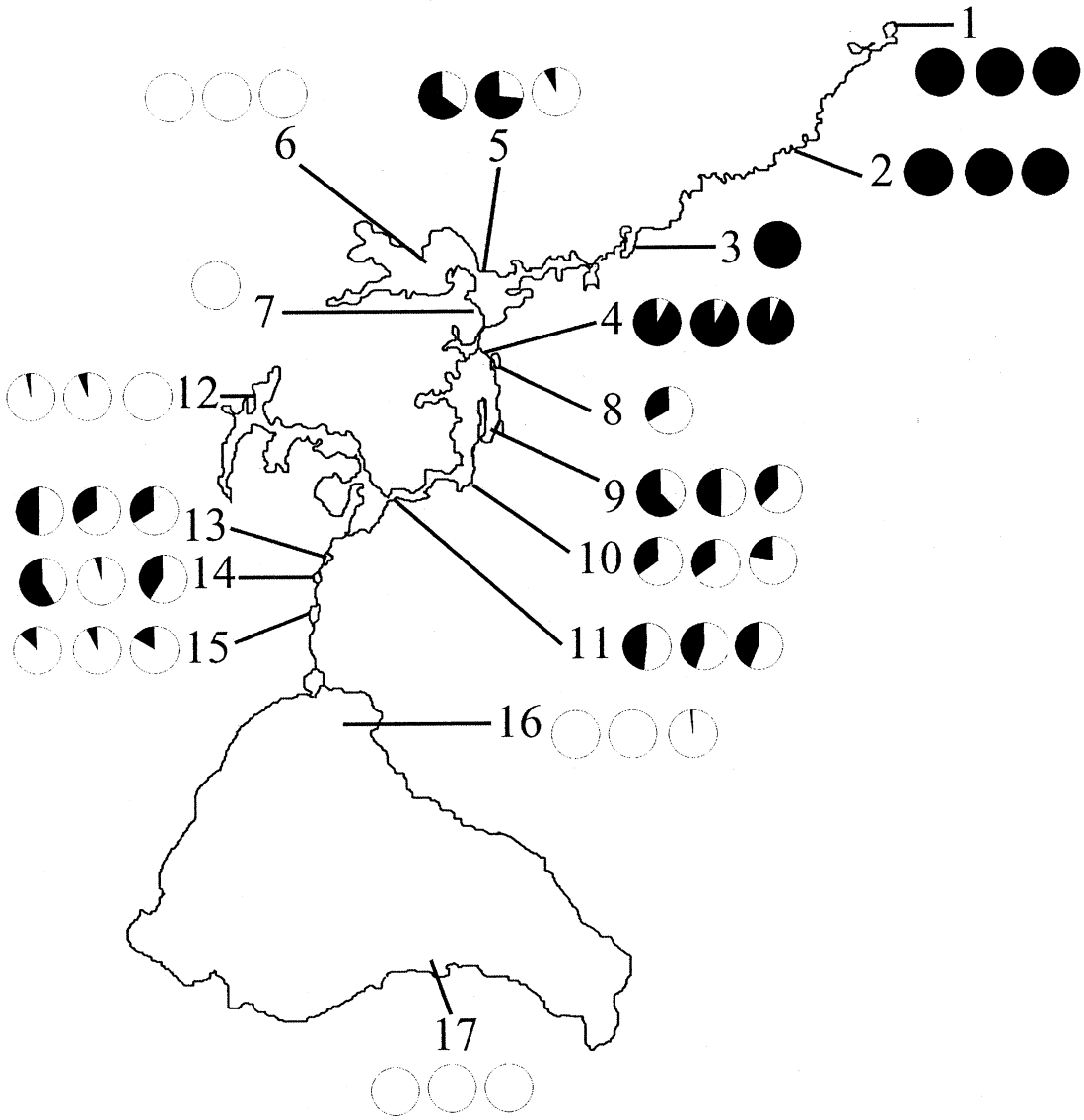

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Figure 3. RAG-1 variation in December 2000, and March and August 2001 across sites CHU 01-17. From left to right pie diagrams represent December, March, and August, respectively. CHU 03, 07, and 08 are represented only by August 2001. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

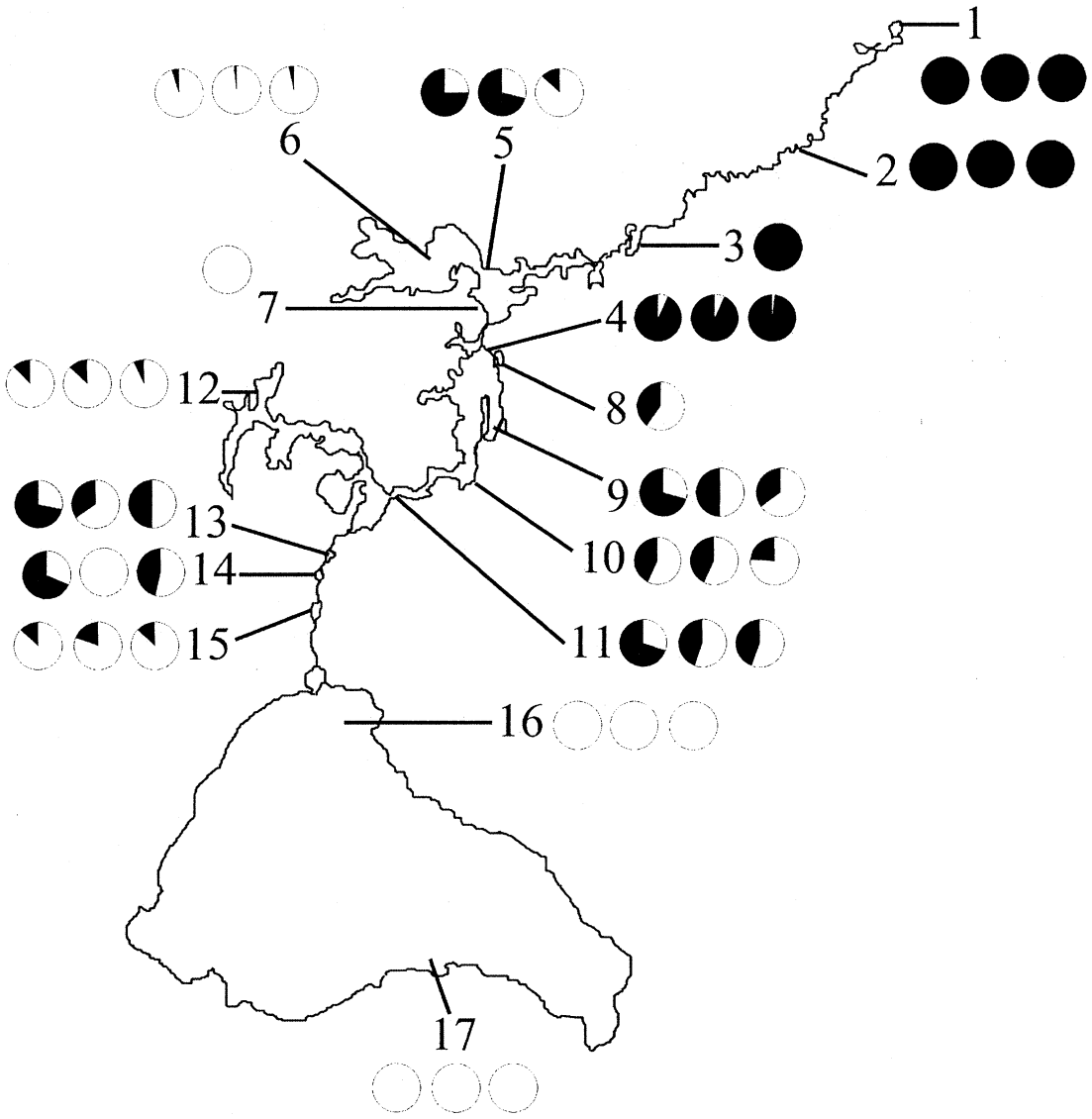

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Figure 4. TPI-B4 variation in December 2000, and March and August 2001 across sites CHU 01-17. From left to right pie diagrams represent December, March, and August, respectively. CHU 03, 07, and 08 are represented only by August 2001. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

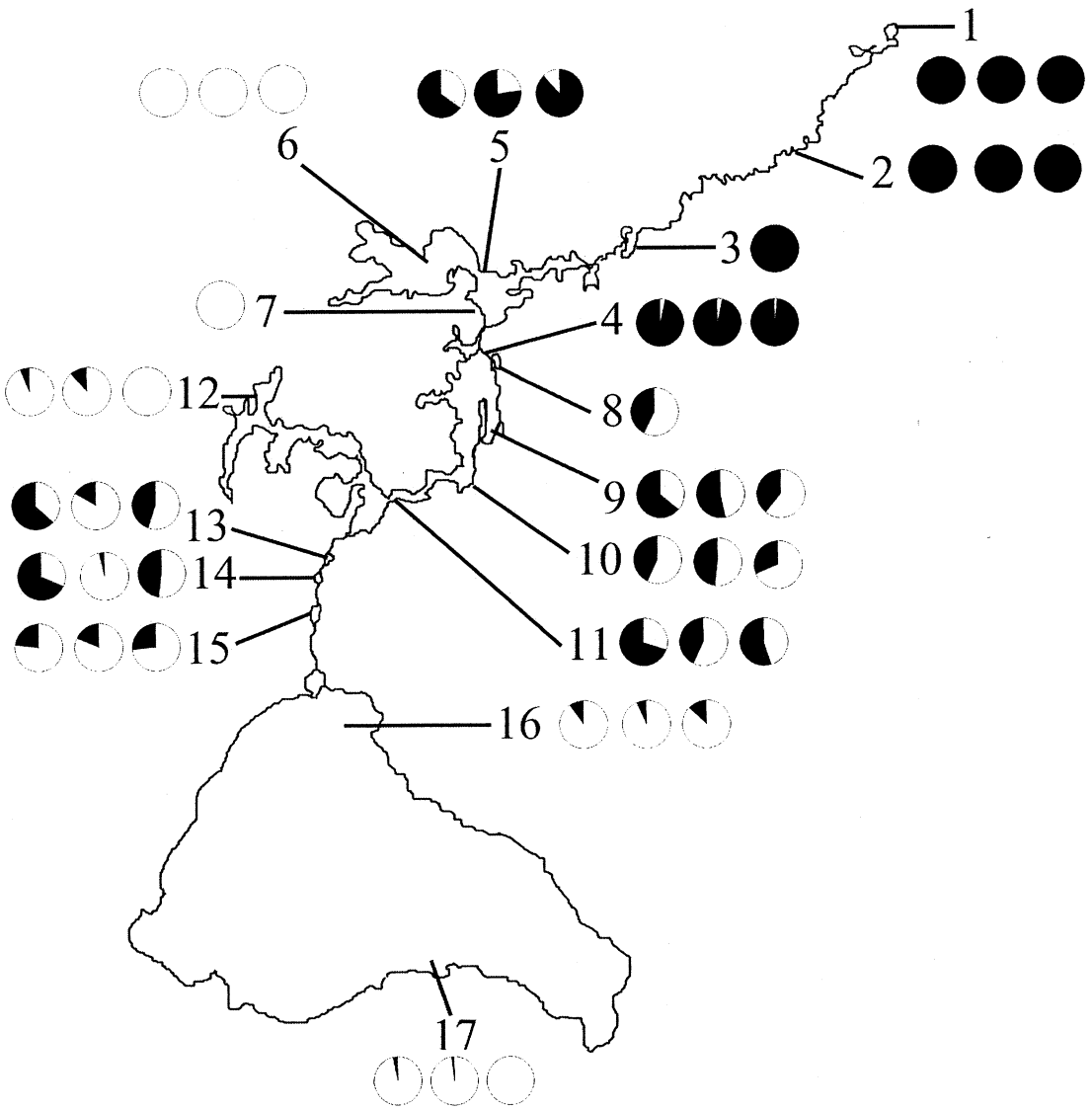

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Figure 5. CK-A7 variation in January, March and August 2002 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

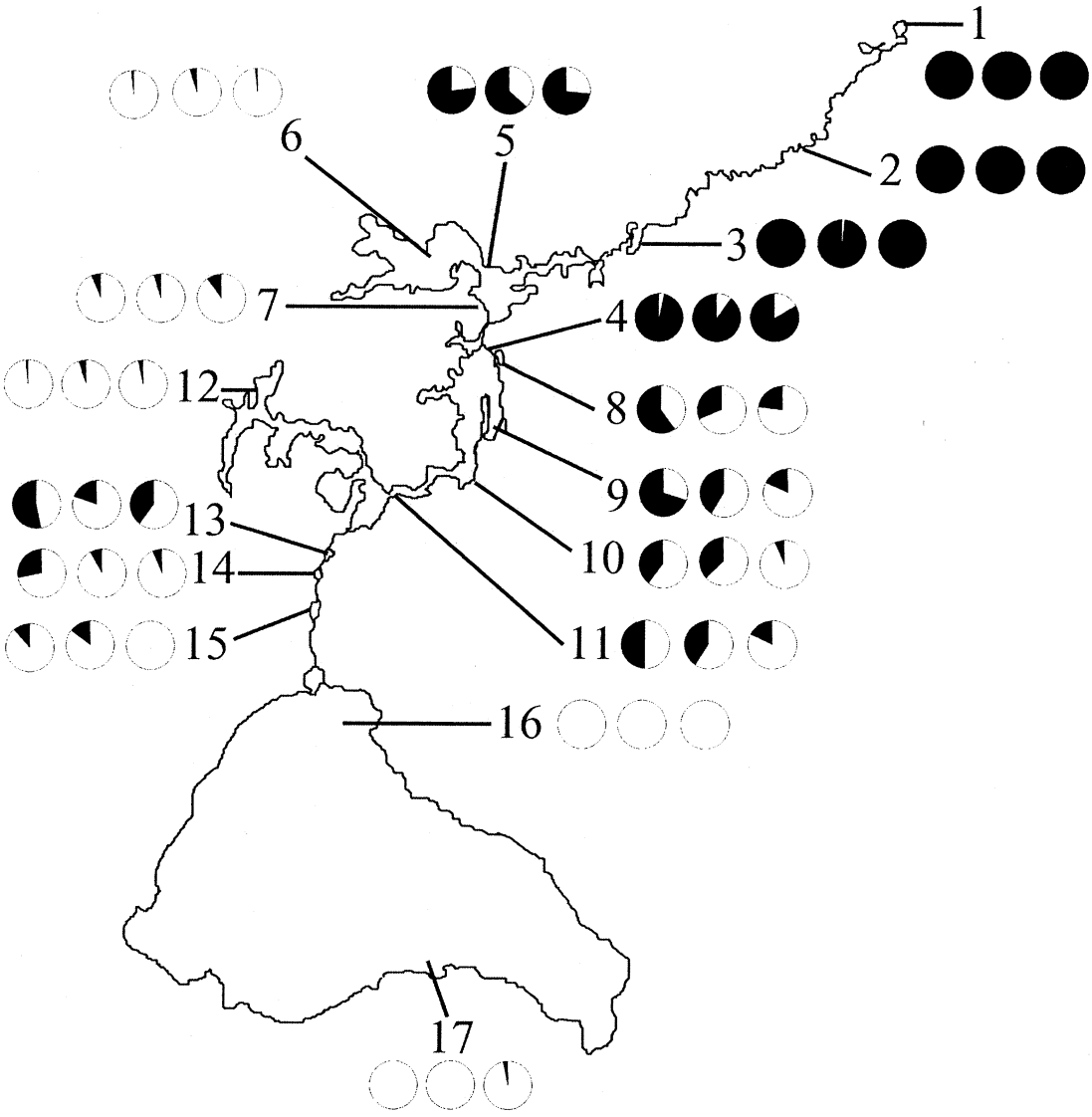

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Figure 6. RAG-1 variation in January, March and August 2002 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

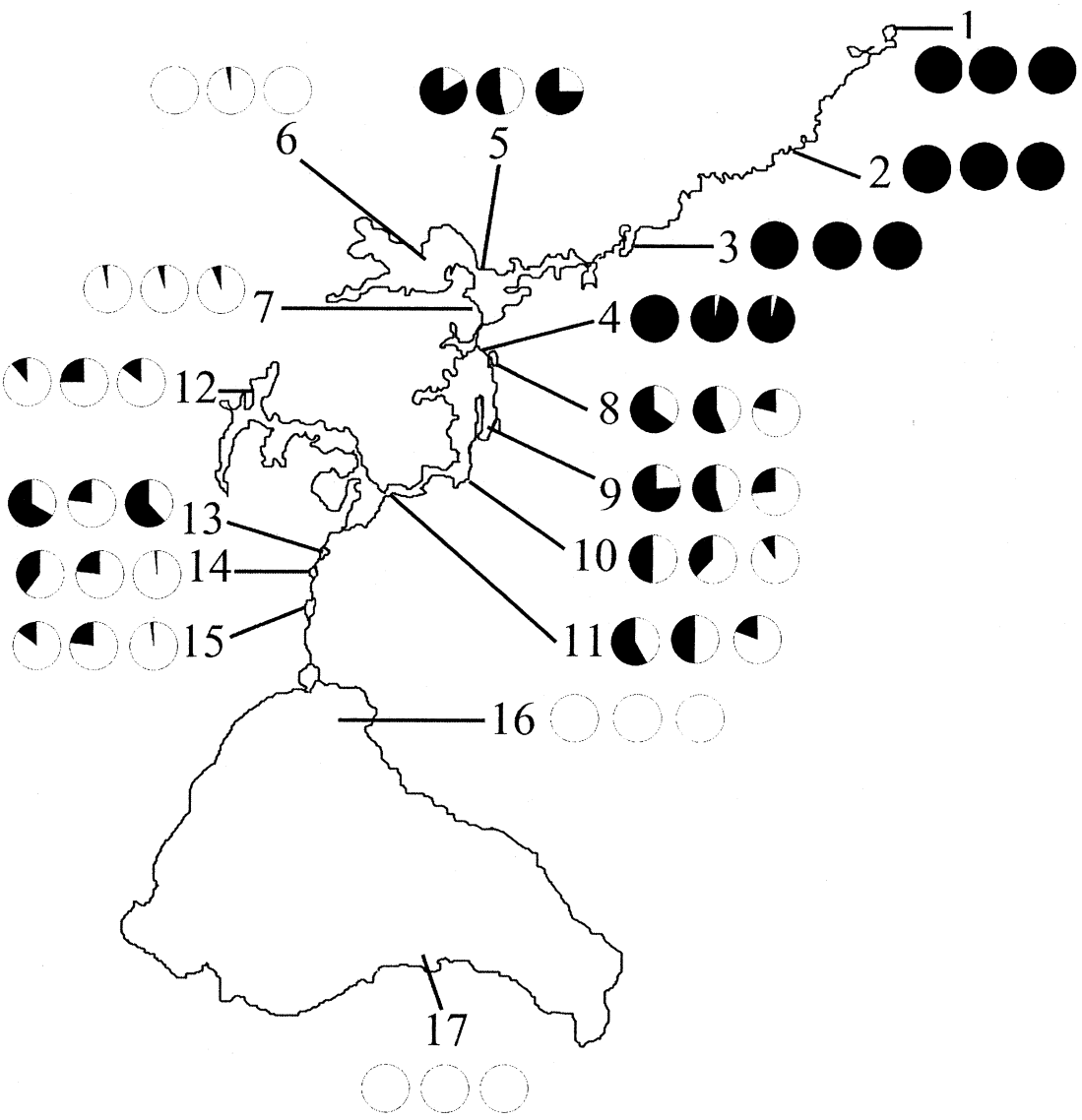

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Figure 7. TPI-B4 variation in January, March and August 2002 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

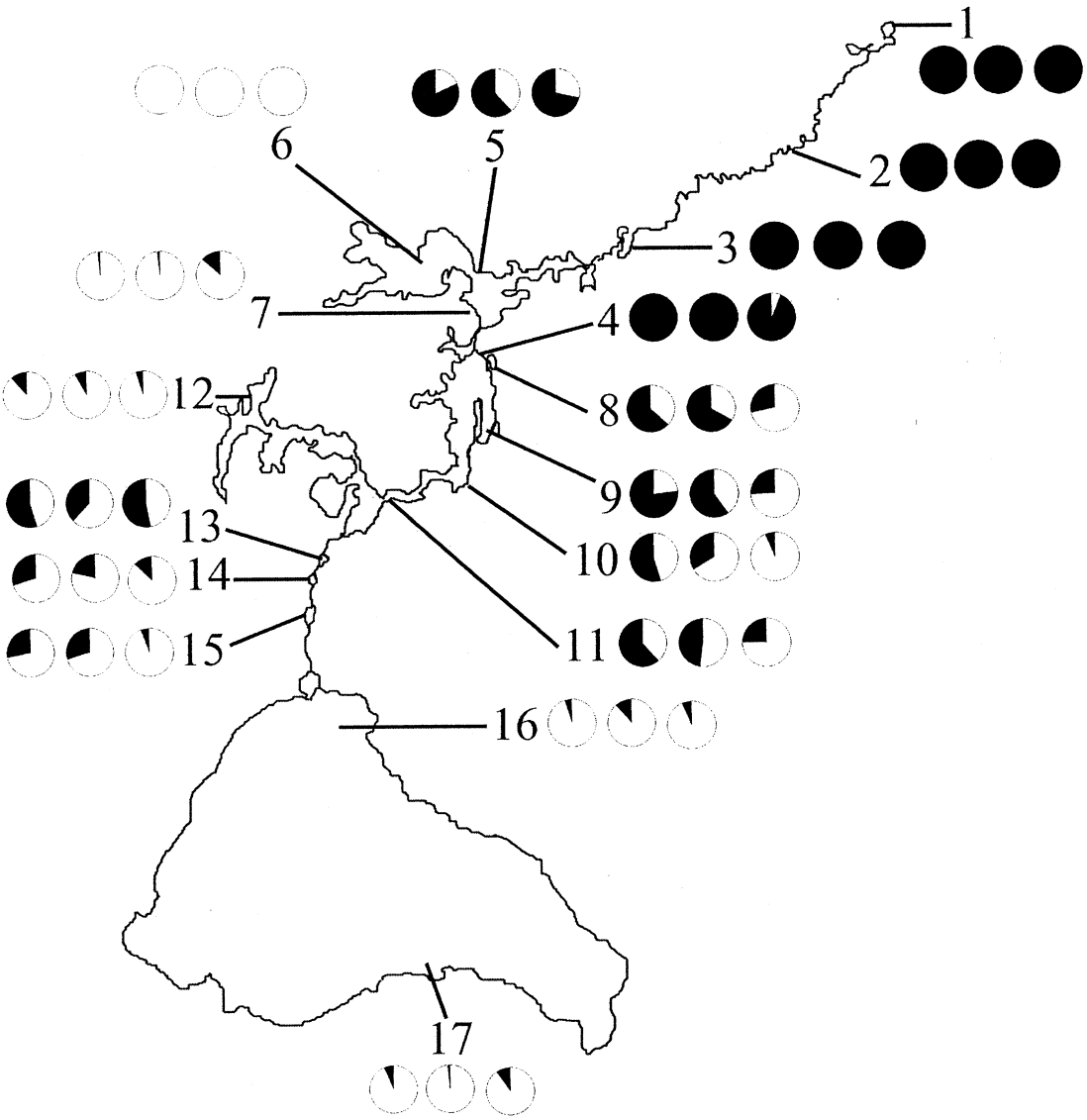

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Figure 8. CK-A7 variation in January, March and August 2003 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

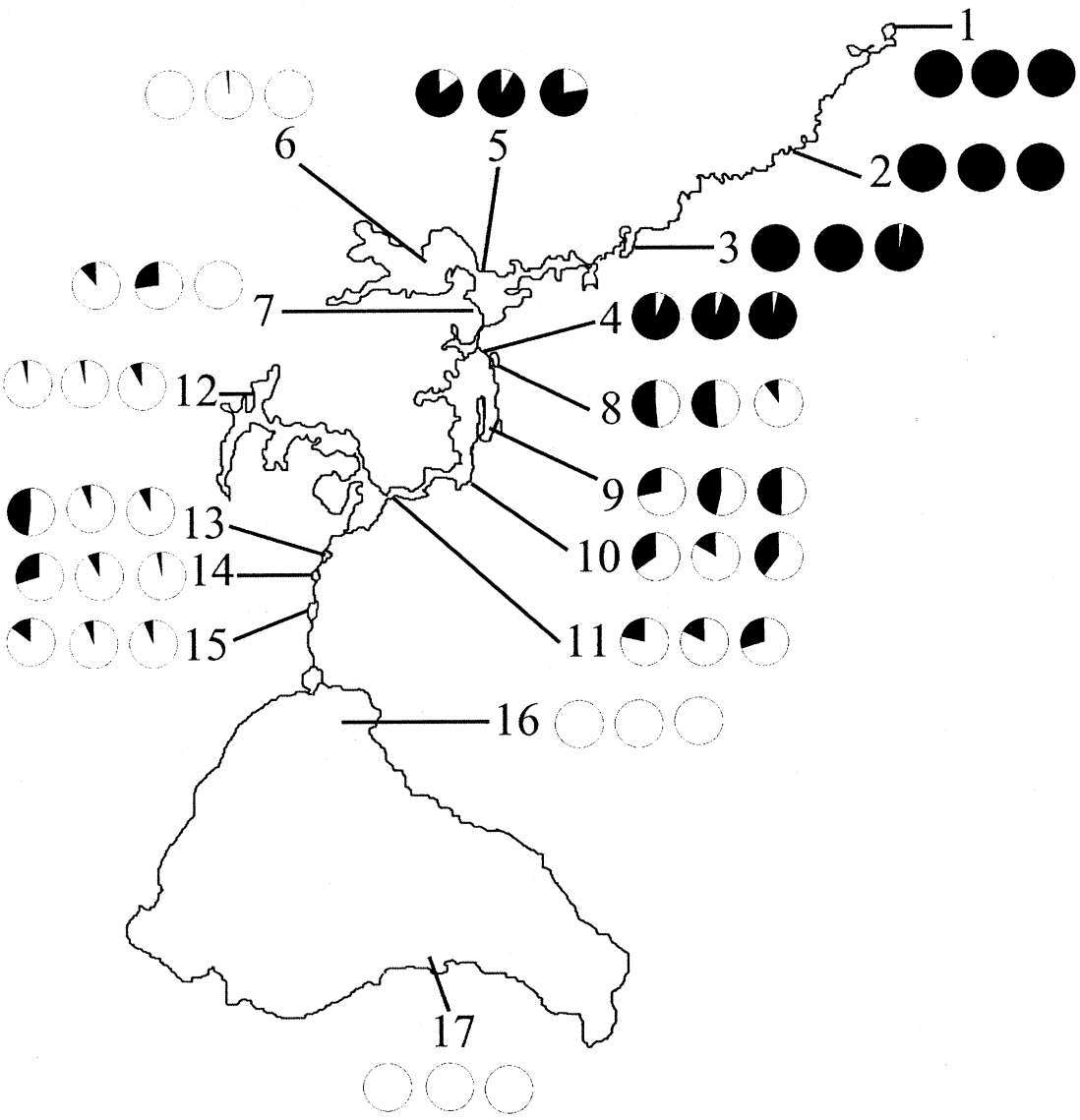

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Figure 9. RAG-1 variation in January, March and August 2003 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

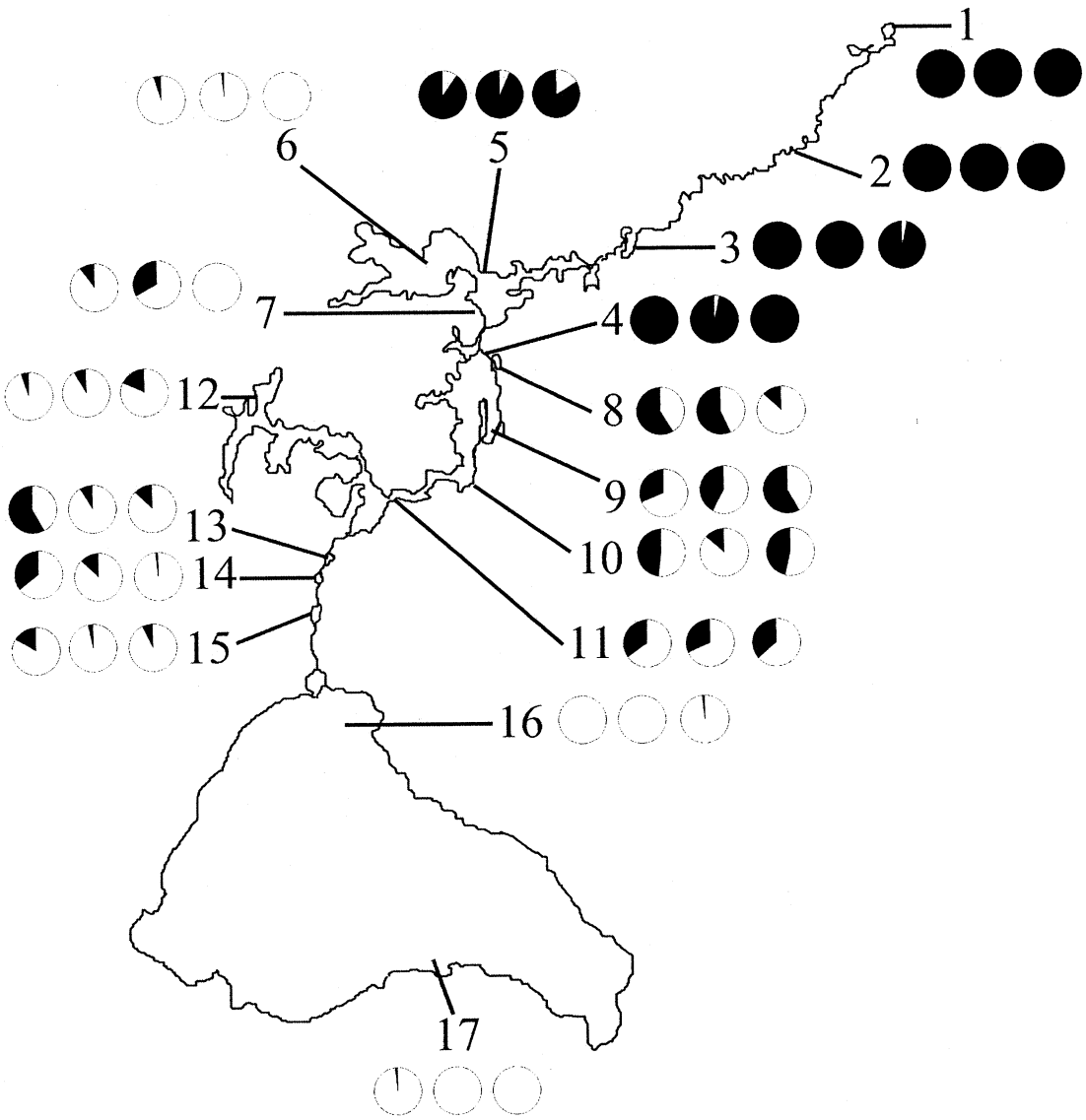

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Figure 10. TPI-B4 variation in January, March and August 2003 across sites CHU 01-17. From left to right pie diagrams represent January, March, and August, respectively. White represents frequency of *Cyprinodon atrorus* alleles and black represents frequency of *C. bifasciatus* alleles.

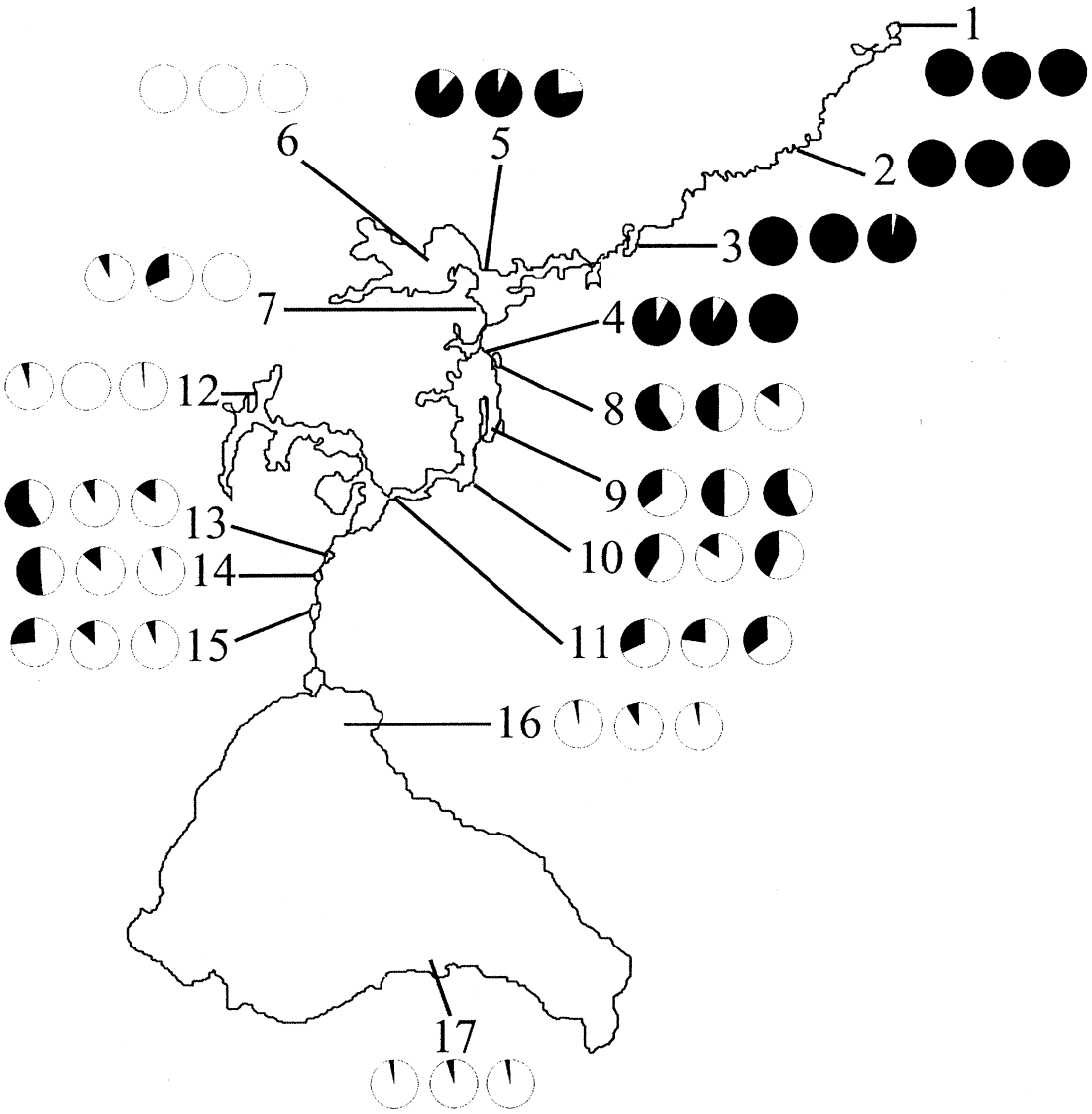

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Figure 11. Seasonal specific conductance (μS) change in the Rio Churince system across years 1-3 and sampling sites CHU01-17. Samples are arranged by habitat type, with sites arranged in the following way: CHU1-3, thermally buffered riverine sites, ;CHU04, thermally buffered laguna, CHU05, 08, 09, 10, and 11, peripheral habitats; CHU13, 14, and 15, lower riverine habitats; CHU07, 12, and 06 peripheral habitats; and CHU16 and 17, terminal habitats.

Specific Conductance (µS)
12000
10000
8000
6000
4000
2000
0
CHU01
CHU02
CHU03
CHU04
CHU05
CHU08
CHU09
CHU10
CHU11
CHU13
CHU14
CHU15
CHU07
CHU12
CHU06
CHU16
CHU17
Dec-00
Mar-01
Aug-01
Jan-02
Mar-02
Aug-02
Jan-03
Mar-03
Aug-03

Figure 12. Seasonal temperature variance in the Rio Churince system across years 1-3 and sampling sites CHU01-17. Samples are arranged by habitat type, with sites arranged in the following way: CHU1-3, thermally buffered riverine sites, ;CHU04, thermally buffered laguna, CHU05, 08, 09, 10, and 11, peripheral habitats; CHU13, 14, and 15, lower riverine habitats; CHU07, 12, and 06 peripheral habitats; and CHU16 and 17, terminal habitats.

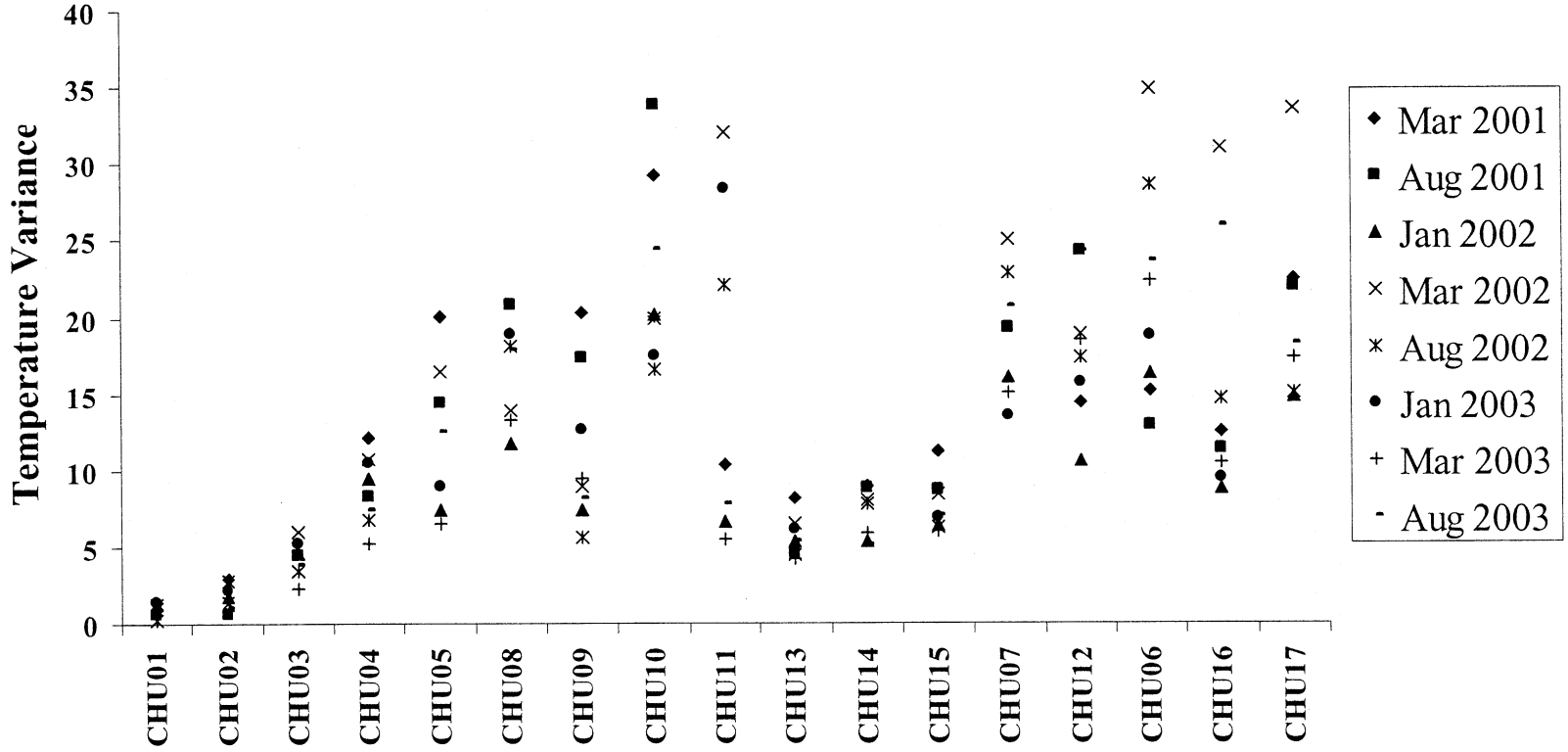

Temperature Variance
40
35
30
25
20
15
10
5
0
CHU01
CHU02
CHU03
CHU04
CHU05
CHU08
CHU09
CHU10
CHU11
CHU13
CHU14
CHU15
CHU07
CHU12
CHU06
CHU16
CHU17
Mar 2001
Aug 2001
Jan 2002
Mar 2002
Aug 2002
Jan 2003
Mar 2003
Aug 2003

Figure 13. Association between *C. bifasciatus* allele frequency at CK-A7 and temperature variance for years 1 through 3.

Association between *C. bifasciatus* allele frequency at CK-A7 and temperature variance for years 1 through 3

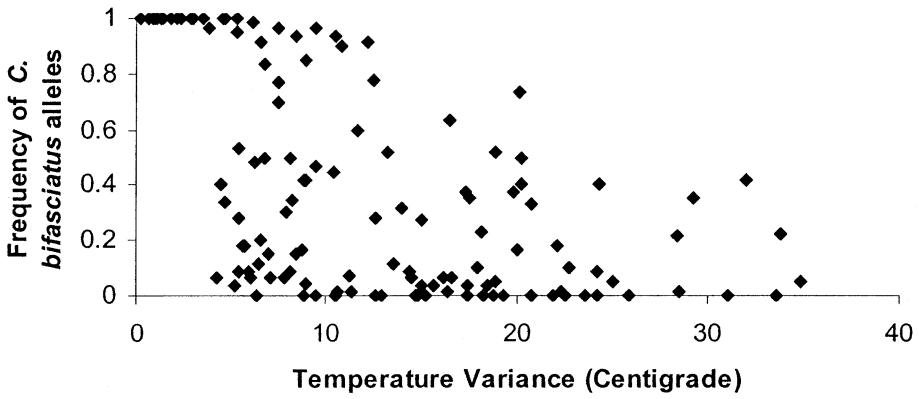


Figure 14. Association between *C. bifasciatus* allele frequency at RAG-1 and temperature variance for years 1 through 3.

Association between *C. bifasciatus* allele frequency at RAG-1 and temperature variance for years 1 through 3

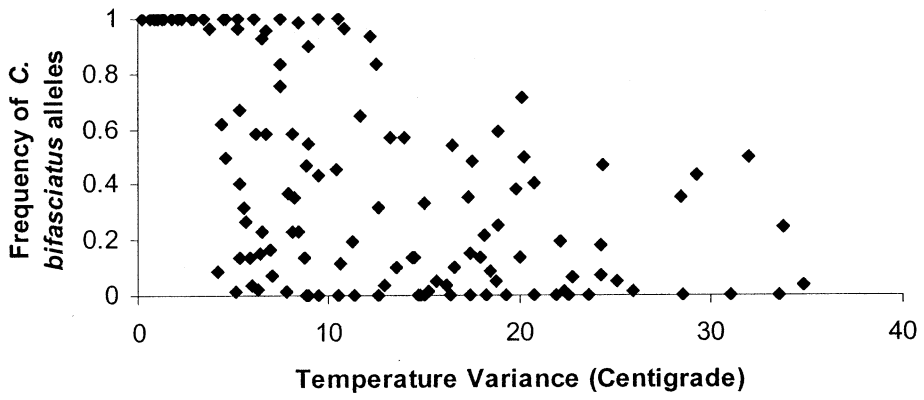


Figure 15. Association between *C. bifasciatus* allele frequency at TPI-B4 and temperature variance for years 1 through 3.

Association between *C. bifasciatus* allele frequency at TPI-B4 and temperature variance for years 1 through 3

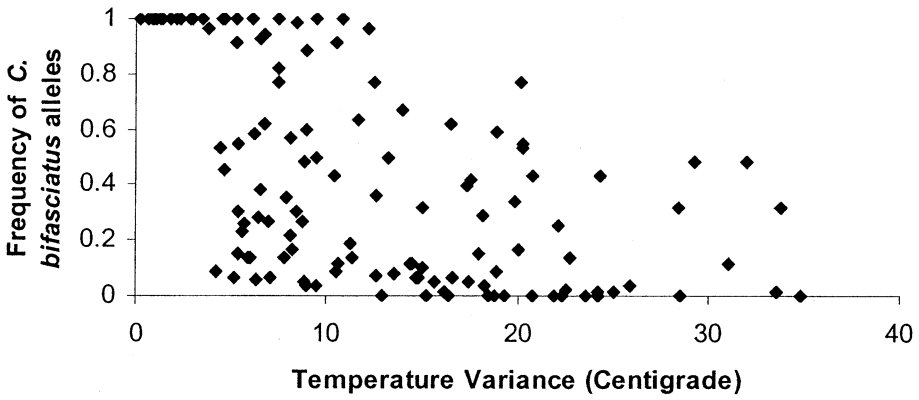


Table 1. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for December 2000. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 1.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	30	-
CHU02	0	0	29	-	0	0	30	-	0	0	30	-
CHU03	-	-	-	-	-	-	-	-	-	-	-	-
CHU04	1	3	26	>0.05	1	2	26	>0.05	0	2	28	>0.05
CHU05	5	11	14	>0.05	2	11	17	>0.05	3	15	12	>0.05
CHU06	30	0	0	-	27	3	0	>0.05	30	0	0	-
CHU07	-	-	-	-	-	-	-	-	-	-	-	-
CHU08	-	-	-	-	-	-	-	-	-	-	-	-
CHU09	5	12	12	>0.05	4	9	16	>0.05	3	15	12	>0.05
CHU10	14	11	5	>0.05	9	16	5	>0.05	9	16	5	>0.05
CHU11	6	19	5	>0.05	5	8	17	>0.05	3	12	15	>0.05
CHU12	28	2	3	>0.05	22	5	1	>0.05	26	4	0	>0.05
CHU13	6	17	6	>0.05	3	11	16	>0.05	3	16	11	>0.05
CHU14	5	15	10	>0.05	5	9	16	>0.05	2	15	13	>0.05
CHU15	23	6	1	>0.05	23	6	1	>0.05	20	6	4	>0.05
CHU16	30	0	0	-	29	0	0	-	24	6	0	>0.05
CHU17	26	0	0	-	26	0	0	-	28	2	0	>0.05

Table 2. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for March 2001. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 2.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	30	-
CHU02	0	0	30	-	0	0	30	-	0	0	30	-
CHU03	-	-	-	-	-	-	-	-	-	-	-	-
CHU04	0	5	25	>0.05	0	4	26	>0.05	0	2	28	>0.05
CHU05	1	10	15	>0.05	2	11	14	>0.05	1	10	17	>0.05
CHU06	30	0	0	-	29	1	0	>0.05	30	0	0	-
CHU07	-	-	-	-	-	-	-	-	-	-	-	-
CHU08	-	-	-	-	-	-	-	-	-	-	-	-
CHU09	7	12	9	>0.05	7	13	7	>0.05	4	18	6	>0.05
CHU10	14	11	5	>0.05	15	4	11	>0.05	11	9	10	>0.05
CHU11	9	14	6	>0.05	6	21	3	>0.05	8	18	4	>0.05
CHU12	26	4	0	>0.05	22	8	0	>0.05	23	7	0	>0.05
CHU13	13	12	4	>0.05	13	13	4	>0.05	15	9	1	>0.05
CHU14	21	2	0	>0.05	30	0	0	-	25	2	0	>0.05
CHU15	24	2	1	>0.05	20	5	3	>0.05	22	3	4	>0.05
CHU16	28	0	0	-	30	0	0	-	25	4	0	>0.05
CHU17	23	0	0	-	30	0	0	-	23	3	0	>0.05

Table 3. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for August 2001. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 3.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	21	-	0	0	30	-
CHU02	0	0	30	-	0	0	22	-	0	0	30	-
CHU03	0	0	28	-	0	0	29	-	0	0	29	>0.05
CHU04	0	4	26	>0.05	0	1	29	>0.05	0	1	29	>0.05
CHU05	26	3	1	>0.05	25	2	3	>0.05	26	1	3	>0.05
CHU06	27	0	0	-	25	2	0	>0.05	26	0	0	-
CHU07	30	0	0	-	25	0	0	-	27	0	0	-
CHU08	13	14	3	>0.05	11	9	8	>0.05	9	15	5	>0.05
CHU09	11	6	7	>0.05	9	13	2	>0.05	10	9	5	>0.05
CHU10	17	11	1	>0.05	16	12	1	>0.05	15	11	4	>0.05
CHU11	8	12	5	>0.05	9	13	6	>0.05	8	10	11	>0.05
CHU12	30	0	0	-	23	6	1	>0.05	30	0	0	-
CHU13	12	13	3	>0.05	12	6	12	>0.05	10	13	7	>0.05
CHU14	12	10	7	>0.05	10	12	8	>0.05	8	15	7	>0.05
CHU15	23	4	3	>0.05	24	4	2	>0.05	20	6	4	>0.05
CHU16	29	1	0	-	30	0	0	-	23	6	1	>0.05
CHU17	30	0	0	-	30	0	0	-	30	0	0	-

Table 4. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for January 2002. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 4.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	30	-
CHU02	0	0	30	-	0	0	30	-	0	0	30	-
CHU03	0	0	30	-	0	0	30	-	0	0	30	-
CHU04	0	2	28	>0.05	0	0	30	-	0	0	30	-
CHU05	1	12	17	>0.05	0	10	20	>0.05	2	7	21	>0.05
CHU06	29	1	0	>0.05	30	0	0	-	30	0	0	-
CHU07	26	4	0	>0.05	28	2	0	>0.05	29	1	0	>0.05
CHU08	3	18	9	>0.05	1	19	10	>0.05	4	14	12	>0.05
CHU09	2	14	14	>0.05	2	10	17	>0.05	0	14	16	>0.05
CHU10	11	14	5	>0.05	10	10	10	>0.05	8	11	11	>0.05
CHU11	8	14	8	>0.05	3	19	8	>0.05	3	17	10	>0.05
CHU12	29	1	0	>0.05	23	7	0	>0.05	23	7	0	>0.05
CHU13	7	14	9	>0.05	5	10	15	>0.05	5	17	8	>0.05
CHU14	17	9	4	>0.05	15	6	9	>0.05	14	14	2	>0.05
CHU15	26	3	1	>0.05	24	3	3	>0.05	16	11	3	>0.05
CHU16	30	0	0	-	30	0	0	-	27	3	0	>0.05
CHU17	30	0	0	-	30	0	0	-	26	4	0	>0.05

Table 5. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for March 2002. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 5.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	27	-	0	0	27	-	0	0	27	-
CHU02	0	0	30	-	0	0	28	-	0	0	30	-
CHU03	0	1	29	>0.05	0	0	30	-	0	0	30	-
CHU04	0	6	24	>0.05	0	2	27	>0.05	0	0	30	-
CHU05	6	10	14	>0.05	9	7	11	>0.05	8	7	15	>0.05
CHU06	27	3	0	>0.05	28	2	0	>0.05	30	0	0	-
CHU07	27	3	0	>0.05	27	3	0	>0.05	29	1	0	>0.05
CHU08	15	11	4	>0.05	5	16	9	>0.05	2	14	11	>0.05
CHU09	9	17	4	>0.05	9	9	12	>0.05	5	14	11	>0.05
CHU10	11	13	4	>0.05	12	12	5	>0.05	14	9	5	>0.05
CHU11	11	12	6	>0.05	7	14	9	>0.05	8	15	7	>0.05
CHU12	27	3	0	>0.05	15	15	0	>0.05	25	5	0	>0.05
CHU13	20	8	2	>0.05	9	8	3	>0.05	13	11	6	>0.05
CHU14	26	3	1	>0.05	20	6	4	>0.05	20	7	3	>0.05
CHU15	21	9	0	>0.05	17	12	1	>0.05	16	10	4	>0.05
CHU16	30	0	0	-	30	0	0	-	25	3	2	>0.05
CHU17	30	0	0	-	30	0	0	-	29	1	0	>0.05

Table 6. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for August 2002. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 6.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	28	-
CHU02	0	0	28	-	0	0	30	-	0	0	30	-
CHU03	0	0	30	-	0	0	29	-	0	0	30	-
CHU04	2	6	22	>0.05	1	0	24	>0.05	0	0	30	-
CHU05	4	8	18	>0.05	3	9	18	>0.05	1	1	23	>0.05
CHU06	29	1	0	>0.05	30	0	0	-	30	0	0	-
CHU07	24	4	1	>0.05	27	2	1	>0.05	25	4	1	>0.05
CHU08	21	4	5	>0.05	19	6	3	>0.05	21	2	7	>0.05
CHU09	23	3	4	>0.05	18	5	5	>0.05	18	7	5	>0.05
CHU10	27	2	1	>0.05	26	2	2	>0.05	27	2	1	>0.05
CHU11	19	3	3	>0.05	20	5	3	>0.05	18	8	4	>0.05
CHU12	27	2	0	>0.05	29	1	0	>0.05	27	3	0	>0.05
CHU13	13	10	7	>0.05	3	16	10	>0.05	6	16	8	>0.05
CHU14	27	2	1	>0.05	28	1	0	>0.05	23	6	1	>0.05
CHU15	30	0	0	-	20	1	0	>0.05	24	5	0	>0.05
CHU16	30	0	0	-	30	0	0	-	26	4	0	>0.05
CHU17	28	2	0	>0.05	30	0	0	-	24	6	0	>0.05

Table 7. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for January 2003. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 7.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	29	-	0	0	30	-	0	0	30	-
CHU02	0	0	29	-	0	0	30	-	0	0	30	-
CHU03	0	0	30	-	0	0	30	-	0	0	30	-
CHU04	1	2	27	>0.05	0	0	30	-	0	5	25	>0.05
CHU05	1	7	22	>0.05	0	6	24	>0.05	1	5	24	>0.05
CHU06	30	0	0	-	27	3	0	>0.05	30	0	0	-
CHU07	25	3	2	>0.05	25	2	2	>0.05	23	2	1	>0.05
CHU08	8	11	9	>0.05	6	11	11	>0.05	6	11	11	>0.05
CHU09	16	11	3	>0.05	17	7	6	>0.05	14	9	6	>0.05
CHU10	16	7	7	>0.05	11	9	10	>0.05	12	11	7	>0.05
CHU11	19	9	2	>0.05	15	9	6	>0.05	16	9	5	>0.05
CHU12	28	2	0	>0.05	26	3	0	>0.05	26	3	0	>0.05
CHU13	11	9	10	>0.05	5	15	10	>0.05	6	12	11	>0.05
CHU14	16	10	4	>0.05	15	6	7	>0.05	8	12	9	>0.05
CHU15	24	3	3	>0.05	22	6	2	>0.05	20	4	6	>0.05
CHU16	30	0	0	-	30	0	0	-	27	2	0	>0.05
CHU17	30	0	0	-	29	1	0	>0.05	28	2	0	>0.05

Table 8. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for March 2003. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 8.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	30	-
CHU02	0	0	30	-	0	0	30	-	0	0	30	-
CHU03	0	0	30	-	0	0	29	-	0	0	30	-
CHU04	0	3	26	>0.05	0	2	27	>0.05	0	5	24	>0.05
CHU05	0	5	24	>0.05	1	2	26	>0.05	0	4	25	>0.05
CHU06	29	1	0	>0.05	28	1	0	>0.05	29	0	0	-
CHU07	18	6	5	>0.05	17	6	7	>0.05	18	5	7	>0.05
CHU08	8	13	9	>0.05	7	11	11	>0.05	9	12	9	>0.05
CHU09	6	19	4	>0.05	8	16	4	>0.05	6	17	6	>0.05
CHU10	21	8	1	>0.05	22	6	1	>0.05	21	8	1	>0.05
CHU11	7	4	0	>0.05	5	5	1	>0.05	6	5	0	>0.05
CHU12	28	2	0	>0.05	24	5	0	>0.05	30	0	0	-
CHU13	27	2	1	>0.05	23	5	0	>0.05	25	5	0	>0.05
CHU14	26	3	1	>0.05	23	6	1	>0.05	23	6	1	>0.05
CHU15	26	4	0	>0.05	29	0	1	>0.05	23	6	1	>0.05
CHU16	30	0	0	-	30	0	0	-	24	5	0	>0.05
CHU17	29	0	0	-	30	0	0	-	26	3	0	>0.05

Table 9. Distribution of CK-A7, RAG-1, and TPI-B4 genotypes and Hardy-Weinberg equilibrium across sites CHU01-17 for August 2003. "A" and "B" alleles represent *Cyprinodon atrorus* and *C. bifasciatus* alleles, respectively. Numbers of each genotype represent the number of occurrences of the genotype for each collection site. HWE (P) denotes p-values for deviation from Hardy-Weinberg equilibrium for each site and locus combination.

Table 9.

	CK			HWE	RAG			HWE	TPI			HWE
Population	AA	AB	BB	P	AA	AB	BB	P	AA	AB	BB	P
CHU01	0	0	30	-	0	0	30	-	0	0	30	-
CHU02	0	0	12	-	0	0	12	-	0	0	12	-
CHU03	1	0	28	>0.05	1	0	28	>0.05	1	0	28	>0.05
CHU04	0	2	27	>0.05	0	0	27	-	0	0	30	-
CHU05	4	5	20	>0.05	4	2	24	>0.05	4	6	20	>0.05
CHU06	30	0	0	-	30	0	0	-	30	0	0	-
CHU07	30	0	0	-	30	0	0	-	28	0	0	-
CHU08	24	4	1	>0.05	23	6	1	>0.05	22	7	0	>0.05
CHU09	6	18	6	>0.05	3	19	8	>0.05	6	14	10	>0.05
CHU10	12	12	6	>0.05	9	14	7	>0.05	8	18	4	>0.05
CHU11	16	10	4	>0.05	11	16	3	>0.05	12	15	3	>0.05
CHU12	25	5	0	>0.05	20	9	1	>0.05	29	1	0	>0.05
CHU13	26	3	1	>0.05	23	6	1	>0.05	23	5	2	>0.05
CHU14	28	2	0	>0.05	29	1	0	>0.05	27	2	1	>0.05
CHU15	26	4	0	>0.05	24	4	0	>0.05	27	2	1	>0.05
CHU16	30	0	0	-	29	1	0	>0.05	28	2	0	-
CHU17	30	0	0	-	30	0	0	-	28	2	0	-

Table 10. Linkage disequilibrium within the Rio Churince hybrid zone for CK-A7, RAG-1, and TPI-B4 for December 2000 and March and August 2001. Dashes indicate sites in which linkage disequilibrium could not be measured. Numbers represent P-values, with bold numbers indicating significant linkage disequilibrium at the alpha equals 0.05 level for the specified pair of loci.

Table 10.

Dec. 2000	CHU01	CHU02	CHU03	CHU04	CHU05	CHU06	CHU07	CHU08	CHU09	CHU10	CHU11	CHU12	CHU13	CHU14	CHU15	CHU16	CHU17
CK-RAG	-	-	-	0.0033	**0.0000**	-	-	-	0.1150	0.0101	0.0037	0.6321	0.1957	0.0046	**0.0000**	-	-
CK-TPI	-	-	-	0.0022	**0.0001**	-	-	-	0.0104	0.0101	0.0273	0.1880	0.1752	0.1149	**0.0000**	-	-
RAG-TPI	-	-	-	0.0050	**0.0000**	-	-	-	0.0108	**0.0000**	**0.0000**	0.2088	0.3260	0.0018	**0.0000**	-	-
March 2001																	
CK-RAG	-	-	-	0.0862	**0.0000**	-	-	-	0.0068	0.0067	0.1794	0.9342	**0.0000**	-	0.0799	-	-
CK-TPI	-	-	-	0.0046	0.0005	-	-	-	0.0034	0.0032	0.3558	**0.0002**	0.4978	-	0.0015	-	-
RAG-TPI	-	-	-	0.1880	0.0003	-	-	-	0.0051	**0.0000**	0.0364	0.0408	0.6828	-	0.0006	-	-
August 2001																	
CK-RAG	-	-	-	0.0384	0.0005	-	-	0.0048	0.0054	0.5807	0.0071	-	**0.0001**	0.0015	**0.0000**	-	-
CK-TPI	-	-	-	0.0192	**0.0001**	-	-	0.0062	**0.0000**	0.0019	0.0595	-	0.0149	0.0015	0.0003	-	-
RAG-TPI	-	-	-	0.0124	**0.0000**	-	-	0.0004	0.0005	0.0690	**0.0001**	-	**0.0001**	0.0006	0.0003	-	-

Table 11. Linkage disequilibrium within the Rio Churince hybrid zone for CK-A7, RAG-1, and TPI-B4 for January, March, and August 2002. Dashes indicate sites in which linkage disequilibrium could not be measured. Numbers represent P-values, with bold numbers indicating significant linkage disequilibrium at the alpha equals 0.05 level for the specified pair of loci.

Table 11.

January 2002	CHU01	CHU02	CHU03	CHU04	CHU05	CHU06	CHU07	CHU08	CHU09	CHU10	CHU11	CHU12	CHU13	CHU14	CHU15	CHU16	CHU17
CK-RAG	-	-	-	-	**0.0001**	-	0.0024	0.4660	0.0193	0.0006	0.1752	0.6168	0.0164	**0.0000**	**0.0002**	-	-
CK-TPI	-	-	-	-	**0.0001**	-	0.0384	0.0194	0.1448	0.0003	**0.0001**	0.6168	0.1770	**0.0000**	0.0013	-	-
RAG-TPI	-	-	-	-	0.0012	-	0.0142	0.0428	0.0274	**0.0000**	0.0095	0.1733	0.0514	**0.0000**	**0.0002**	-	-
March 2002																	
CK-RAG	-	-	-	-	**0.0000**	-	0.6477	0.5380	0.0064	0.0014	0.0105	0.5287	**0.0000**	0.0093	0.0153	-	-
CK-TPI	-	-	-	-	**0.0000**	-	0.6477	0.4984	0.0047	0.0251	**0.0000**	0.0351	0.0004	0.0978	0.0167	-	-
RAG-TPI	-	-	-	-	**0.0000**	-	0.6477	0.0005	0.0042	**0.0001**	**0.0007**	0.0038	**0.0000**	**0.0000**	0.0012	-	-
August 2002																	
CK-RAG	-	-	-	0.0194	**0.0001**	-	0.0073	**0.0000**	**0.0000**	**0.0000**	0.0016	0.3851	0.0885	0.8085	-	-	-
CK-TPI	-	-	-	0.0045	0.0013	-	0.0390	**0.0000**	**0.0001**	0.2213	**0.0002**	0.2890	0.6465	0.0122	-	-	-
RAG-TPI	-	-	-	0.0159	0.0050	-	**0.0000**	**0.0000**	**0.0000**	0.0311	0.0434	0.8940	0.3474	0.1002	-	-	-

Table 12. Linkage disequilibrium within the Rio Churince hybrid zone for CK-A7, RAG-1, and TPI-B4 for January, March, and August 2003. Dashes indicate sites in which linkage disequilibrium could not be measured. Numbers represent P-values, with bold numbers indicating significant linkage disequilibrium at the alpha equals 0.05 level for the specified pair of loci.

Table 12.

January 2003	CHU01	CHU02	CHU03	CHU04	CHU05	CHU06	CHU07	CHU08	CHU09	CHU10	CHU11	CHU12	CHU13	CHU14	CHU15	CHU16	CHU17
CK-RAG	-	-	-	-	-	-	**0.0000**	0.0027	0.0008	**0.0000**	0.0029	0.8377	0.0950	**0.0002**	0.0005	-	-
CK-TPI	-	-	-	-	-	-	**0.0000**	**0.0000**	0.0011	**0.0001**	**0.0001**	0.0042	0.0573	0.0172	0.0008	-	-
RAG-TPI	-	-	-	-	-	-	**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	0.9231	0.0006	**0.0000**	0.0027	-	-
March 2003																	
CK-RAG	-	-	-	0.1316	0.0018	-	**0.0000**	**0.0000**	**0.0000**	0.0026	0.0164	-	0.1042	0.2264	0.0830	-	-
CK-TPI	-	-	-	0.0004	**0.0000**	-	**0.0000**	**0.0000**	**0.0000**	0.0113	0.0013	-	0.6439	0.0363	0.0122	-	-
RAG-TPI	-	-	-	0.2637	0.0006	-	**0.0000**	**0.0000**	0.0214	0.0269	0.0019	-	0.5338	**0.0001**	0.2023	-	-
August 2003																	
CK-RAG	-	-	-	-	**0.0000**	-	-	0.0007	0.0025	0.0009	0.0082	0.0252	0.0010	0.7937	0.0338	-	-
CK-TPI	-	-	-	-	**0.0000**	-	-	0.0608	0.0008	0.0110	0.0316	0.0518	0.0010	0.0011	0.0216	-	-
RAG-TPI	-	-	-	-	**0.0000**	-	-	0.0050	**0.0000**	0.0003	0.0117	0.1489	0.0186	0.7091	0.0612	-	-

Table 13. Overall numbers of each multi-locus genotype observed in the Rio Churince hybrid zone. "A" alleles represent *Cyprinodon atrorus* alleles and "B" alleles represent *C. bifasciatus* alleles. Question marks represent missing allelic assignment.

Table 13.

CK-A7	RAG-1	TPI-B4	Composite #	Number of occurances	CK-A7	RAG-1	TPI-B4	Composite #	Number of occurances
??	??	AA	1	2	AB	??	AB	28	5
??	??	AB	2	1	AB	??	BB	29	4
??	??	BB	3	1	AB	AA	??	30	3
??	AA	??	4	3	AB	AA	AA	31	52
??	AA	AA	5	25	AB	AA	AB	32	61
??	AA	AB	6	4	AB	AA	BB	33	8
??	AA	BB	7	1	AB	AB	??	34	4
??	AB	AA	8	1	AB	AB	AA	35	54
??	AB	AB	9	5	AB	AB	AB	36	226
??	AB	BB	10	1	AB	AB	BB	37	75
??	BB	??	11	1	AB	BB	??	38	2
??	BB	AB	12	1	AB	BB	AA	39	8
??	BB	BB	13	12	AB	BB	AB	40	82
AA	??	??	14	4	AB	BB	BB	41	123
AA	??	AA	15	30	BB	??	??	42	2
AA	??	AB	16	4	BB	??	AB	43	3
AA	AA	??	17	18	BB	??	BB	44	33
AA	AA	AA	18	1686	BB	AA	AA	45	1
AA	AA	AB	19	168	BB	AA	AB	46	9
AA	AA	BB	20	11	BB	AB	AA	47	6
AA	AB	AA	21	123	BB	AB	AB	48	50
AA	AB	AB	22	93	BB	AB	BB	49	51
AA	AB	BB	23	24	BB	BB	??	50	12
AA	BB	AA	24	6	BB	BB	AA	51	4
AA	BB	AB	25	31	BB	BB	AB	52	66
AA	BB	BB	26	25	BB	BB	BB	53	1119
AB	??	AA	27	1					

Table 14. Multiple linear regression results for test of correlation between specific conductance, temperature, and frequency of *Cyprinodon atrorus* CK-A7 alleles. Status refers to whether the term (specific conductance or temperature) remained in the final model or was removed. Removed terms did not significantly correlate with frequency of *C. atrorus* alleles, whereas terms that remained in the model were significant predictors of *C. atrorus* allele frequency.

Table 14.
Correlation between environmental variation and frequency of *C. atrorus*-specific CK-A7 alleles

Date	Variable	Status	P	R^2
Dec-00	Sp. Cond.	In Model	0.104	0.222
Mar-01	Sp. Cond.	In Model	0.100	0.209
	Temp. Var.	Removed from Model	-	-
Aug-01	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.006**	0.430
Jan-02	Sp. Cond.	In Model	0.070	
	Temp. Var.	In Model	0.060	0.505
Mar-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.003**	0.459
Aug-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.006**	0.427
Jan-03	Sp. Cond.	In Model	0.073	
	Temp. Var.	In Model	**0.036**	0.504
Mar-03	Sp. Cond.	In Model	0.133	
	Temp. Var.	In Model	**0.013**	0.569
Aug-03	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.004**	0.433

Table 15. Multiple linear regression results for test of correlation between specific conductance, temperature, and frequency of *Cyprinodon atrorus* RAG-1 alleles. Status refers to whether the term (specific conductance or temperature) remained in the final model or was removed. Removed terms did not significantly correlate with frequency of *C. atrorus* alleles, whereas terms that remained in the model were significant predictors of *C. atrorus* allele frequency.

Table 15.
Correlation between environmental variation and frequency of *C. atrorus*-specific RAG-1 alleles

Date	Variable	Status	P	R^2
Dec-00	Sp. Cond.	In Model	**0.045**	0.295
Mar-01	Sp. Cond.	In Model	0.105	0.204
	Temp. Var.	Removed from Model	-	-
Aug-01	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.008**	0.409
Jan-02	Sp. Cond.	In Model	0.102	
	Temp. Var.	In Model	0.051	0.491
Mar-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.001**	0.535
Aug-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.003**	0.486
Jan-03	Sp. Cond.	In Model	0.058	
	Temp. Var.	In Model	0.084	0.461
Mar-03	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.005**	0.421
Aug-03	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.008**	0.381

Table 16. Multiple linear regression results for test of correlation between specific conductance, temperature, and frequency of *Cyprinodon atrorus* TPI-B4 alleles. Status refers to whether the term (specific conductance or temperature) remained in the final model or was removed. Removed terms did not significantly correlate with frequency of *C. atrorus* alleles, whereas terms that remained in the model were significant predictors of *C. atrorus* allele frequency.

Table 16.
Correlation between environmental variation and frequency of *C. atrorus*-specific TPI-B4 alleles

Date	Variable	Status	P	R^2
Dec-00	Sp. Cond.	In Model	0.094	0.216
Mar-01	Sp. Cond.	In Model	0.141	0.171
	Temp. Var.	Removed from Model	-	-
Aug-01	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.005**	0.436
Jan-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.008**	0.384
Mar-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.001**	0.536
Aug-02	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.001**	0.534
Jan-03	Sp. Cond.	In Model	0.091	
	Temp. Var.	In Model	**0.033**	0.497
Mar-03	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.002**	0.499
Aug-03	Sp. Cond.	Removed from Model	-	-
	Temp. Var.	In Model	**0.007**	0.416

Table 17. Distribution of significant seasonal changes in *Cyprinodon bifasciatus* allele frequency (CK-A7) at sites CHU01-17 in the Rio Churince system. Initial Freq (B) and Final Freq (B) equal the initial and final frequencies of *C. bifasciatus* specific alleles at each site between the two measured dates. Gadj χ^2 Value is the Williams corrected G statistic. "Y" and "N" indicate whether allele frequency change met expectations of the respective hypotheses.

Table 17. CK-A7 Allele Frequency Change Across Seasons

Date	Site	Habitat	Initial Freq. (B)	Final Freq. (B)	Gadj X2 value (1*df*)	P	Prediction	Fit Expectation
Dec 2000-Mar 2001	CHU14	Riverine	0.583	0.043	38.545	<0.01	1	Y
Jan-Mar 2002	CHU13	Riverine	0.533	0.200	14.558	<0.01	1	Y
Jan-Mar 2002	CHU14	Riverine	0.283	0.083	9.195	<0.01	1	Y
Jan-Mar 2003	CHU13	Riverine	0.483	0.067	28.186	<0.01	1	Y
Jan-Mar 2003	CHU14	Riverine	0.300	0.083	9.335	<0.01	1	Y
Mar-Aug 2001	CHU14	Riverine	0.043	0.414	21.386	<0.01	1	Y
Mar-Aug 2002	CHU13	Riverine	0.200	0.400	5.708	<0.05	1	Y
Mar-Aug 2002	CHU15	Riverine	0.150	0.000	10.108	<0.01	1	N
Jan-Mar 2002	CHU08	Peripheral	0.600	0.317	9.717	<0.01	2	Y
Jan-Mar 2002	CHU09	Peripheral	0.700	0.417	9.787	<0.01	2	Y
Jan-Mar 2003	CHU07	Peripheral	0.117	0.276	4.588	<0.05	2	N
Jan-Mar 2003	CHU09	Peripheral	0.283	0.466	4.156	<0.05	2	N
Jan-Mar 2003	CHU10	Peripheral	0.350	0.167	5.260	<0.05	2	Y
Mar-Aug 2001	CHU05	Peripheral	0.731	0.083	53.400	<0.01	2	Y
Mar-Aug 2002	CHU09	Peripheral	0.417	0.183	7.813	<0.01	2	Y
Mar-Aug 2002	CHU10	Peripheral	0.375	0.067	17.064	<0.01	2	Y
Mar-Aug 2002	CHU11	Peripheral	0.414	0.180	7.015	<0.01	2	Y
Mar-Aug 2003	CHU05	Peripheral	0.914	0.776	4.216	<0.05	2	Y
Mar-Aug 2003	CHU07	Peripheral	0.276	0.000	21.536	<0.01	2	Y
Mar-Aug 2003	CHU08	Peripheral	0.517	0.103	24.699	<0.01	2	Y
Mar-Aug 2003	CHU10	Peripheral	0.167	0.400	8.097	<0.01	2	N

Table 18. Distribution of significant seasonal changes in *Cyprinodon bifasciatus* allele frequency (RAG-1) at sites CHU01-17 in the Rio Churince system. Initial Freq (B) and Final Freq (B) equal the initial and final frequencies of *C. bifasciatus* specific alleles at each site between the two measured dates. Gadj χ^2 Value is the Williams corrected G statistic. "Y" and "N" indicate whether allele frequency change met expectations of the respective hypotheses.

Table 18. RAG-1 Allele Frequency Change Across Seasons

Date	Site	Habitat	Initial Freq. (B)	Final Freq. (B)	Gadj X2 value (1*df*)	P	Prediction	Fit Expectation
Dec 2000-Mar 2001	CHU13	Riverine	0.717	0.350	16.393	<0.01	1	Y
Dec 2000-Mar 2001	CHU14	Riverine	0.683	0.000	73.807	<0.01	1	Y
Jan-Mar 2002	CHU13	Riverine	0.667	0.233	23.284	<0.01	1	Y
Jan-Mar 2003	CHU13	Riverine	0.583	0.089	33.750	<0.01	1	Y
Jan-Mar 2003	CHU14	Riverine	0.357	0.133	7.730	<0.01	1	Y
Jan-Mar 2003	CHU15	Riverine	0.167	0.033	6.156	<0.05	1	Y
Mar-Aug 2001	CHU14	Riverine	0.000	0.467	42.918	<0.01	1	Y
Mar-Aug 2002	CHU13	Riverine	0.233	0.621	18.400	<0.01	1	Y
Mar-Aug 2002	CHU14	Riverine	0.233	0.017	12.944	<0.01	1	N
Mar-Aug 2003	CHU14	Riverine	0.133	0.017	5.566	<0.05	1	N
Mar-Aug 2002	CHU15	Riverine	0.233	0.024	10.178	<0.01	1	N
Dec 2000-Mar 2001	CHU09	Peripheral	0.707	0.500	4.982	<0.05	2	Y
Dec 2000-Mar 2001	CHU11	Peripheral	0.700	0.450	7.666	<0.01	2	Y
Jan-Mar 2002	CHU05	Peripheral	0.833	0.537	11.783	<0.01	2	Y
Jan-Mar 2002	CHU09	Peripheral	0.759	0.550	5.655	<0.05	2	Y
Jan-Mar 2003	CHU07	Peripheral	0.103	0.333	8.968	<0.01	2	N
Jan-Mar 2003	CHU10	Peripheral	0.484	0.138	16.863	<0.01	2	Y
Mar-Aug 2001	CHU05	Peripheral	0.722	0.133	42.987	<0.01	2	Y
Mar-Aug 2001	CHU10	Peripheral	0.433	0.241	4.837	<0.05	2	Y
Mar-Aug 2002	CHU05	Peripheral	0.537	0.750	5.614	<0.05	2	N
Mar-Aug 2002	CHU08	Peripheral	0.567	0.214	15.298	<0.01	2	Y
Mar-Aug 2002	CHU09	Peripheral	0.550	0.268	9.554	<0.01	2	Y
Mar-Aug 2002	CHU10	Peripheral	0.379	0.100	13.060	<0.01	2	Y
Mar-Aug 2002	CHU11	Peripheral	0.500	0.196	11.486	<0.01	2	Y
Mar-Aug 2003	CHU07	Peripheral	0.333	0.000	27.690	<0.01	2	Y
Mar-Aug 2003	CHU08	Peripheral	0.569	0.133	25.633	<0.01	2	Y
Mar-Aug 2003	CHU10	Peripheral	0.138	0.467	15.474	<0.01	2	N

Table 19. Distribution of significant seasonal changes in *Cyprinodon bifasciatus* allele frequency (TPI-B4) at sites CHU01-17 in the Rio Churince system. Initial Freq (B) and Final Freq (B) equal the initial and final frequencies of *C.bifasciatus* specific alleles at each site between the two measured dates. Gadj χ^2 Value is the Williams corrected G statistic. "Y" and "N" indicate whether allele frequency change met expectations of the respective hypotheses.

Table 19. TPI-B4 Allele Frequency Change Across Seasons

Date	Site	Habitat	Initial Freq. (B)	Final Freq. (B)	Gadj X2 value (1*df*)	P	Prediction	Fit Expectation
Dec 2000-Mar 2001	CHU13	Riverine	0.633	0.183	25.932	<0.01	1	Y
Dec 2000-Mar 2001	CHU14	Riverine	0.683	0.037	58.227	<0.01	1	Y
Jan-Mar 2003	CHU13	Riverine	0.583	0.083	36.310	<0.01	1	Y
Jan-Mar 2003	CHU14	Riverine	0.517	0.133	20.532	<0.01	1	Y
Mar-Aug 2001	CHU13	Riverine	0.183	0.450	9.944	<0.01	1	Y
Mar-Aug 2001	CHU14	Riverine	0.037	0.483	32.618	<0.01	1	Y
Mar-Aug 2002	CHU15	Riverine	0.300	0.087	8.841	<0.01	1	N
Mar-Aug 2003	CHU04	Laguna	0.914	1.000	4.866	<0.05	1	Y
Dec 2000-Mar 2001	CHU11	Peripheral	0.700	0.433	8.693	<0.01	2	Y
Jan-Mar 2002	CHU05	Peripheral	0.817	0.617	5.911	<0.05	2	Y
Jan-Mar 2002	CHU10	Peripheral	0.550	0.339	5.179	<0.05	2	Y
Jan-Mar 2003	CHU07	Peripheral	0.077	0.317	9.872	<0.01	2	N
Jan-Mar 2003	CHU10	Peripheral	0.417	0.167	9.156	<0.01	2	Y
Mar-Aug 2001	CHU05	Peripheral	0.778	0.117	54.608	<0.01	2	Y
Mar-Aug 2001	CHU12	Peripheral	0.117	0.000	7.320	<0.01	2	Y
Mar-Aug 2002	CHU08	Peripheral	0.700	0.267	23.040	<0.01	2	Y
Mar-Aug 2002	CHU09	Peripheral	0.600	0.283	12.272	<0.01	2	Y
Mar-Aug 2002	CHU10	Peripheral	0.339	0.067	14.081	<0.01	2	Y
Mar-Aug 2002	CHU11	Peripheral	0.483	0.267	5.993	<0.05	2	Y
Mar-Aug 2003	CHU05	Peripheral	0.931	0.767	6.310	<0.05	2	Y
Mar-Aug 2003	CHU07	Peripheral	0.317	0.000	24.582	<0.01	2	Y
Mar-Aug 2003	CHU08	Peripheral	0.500	0.121	20.555	<0.01	2	Y
Mar-Aug 2003	CHU10	Peripheral	0.167	0.433	10.271	<0.01	2	N

Table 20. Variation in cline width and selection coefficients across seasons and years in the Rio Churince system for CK-A7. Upper boundary represents the upper limit of the cline in allele frequency (20 percent or less *C. atrorus* specific allele frequency). Lower boundary represents the lower cline limit (80 percent or less *C. atrorus* allele frequency). In December and March of year 1 CHU08 was not sampled, thus cline width during these sampling periods may have been artificially contracted as in the remaining sample dates CHU08 consistently set the upper limit of the cline.

Table 20. Variation in cline width and selection coefficients across seasons and years for CK-A7

Year 1

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
December	CHU09	CHU14	0.62	0.21
March	CHU09	CHU13	0.52	0.30
August	CHU08	CHU14	0.82	0.12

Year 2

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU14	0.82	0.12
March	CHU08	CHU13	0.72	0.15
August	CHU08	CHU13	0.72	0.15

Year 3

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU14	0.82	0.12
March	CHU08	CHU11	0.52	0.30
August	CHU08	CHU11	0.52	0.30

Table 21. Variation in cline width and selection coefficients across seasons and years in the Rio Churince system for RAG-1. Upper boundary represents the upper limit of the cline in allele frequency (20 percent or less *C. atrorus* specific allele frequency). Lower boundary represents the lower cline limit (80 percent or less *C. atrorus* allele frequency). In December and March of year 1 CHU08 was not sampled, thus cline width during these sampling periods may have been artificially contracted as in the remaining sample dates CHU08 consistently set the upper limit of the cline.

Table 21. Variation in cline width and selection coefficients across seasons and years for RAG-1

Year 1

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
December	CHU09	CHU14	0.62	0.21
March	CHU09	CHU13	0.52	0.30
August	CHU08	CHU14	0.82	0.12

Year 2

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU14	0.82	0.12
March	CHU08	CHU15	0.93	0.09
August	CHU08	CHU13	0.72	0.15

Year 3

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU14	0.82	0.12
March	CHU08	CHU11	0.52	0.30
August	CHU08	CHU11	0.52	0.30

Table 22. Variation in cline width and selection coefficients across seasons and years in the Rio Churince system for TPI-B4. Upper boundary represents the upper limit of the cline in allele frequency (20 percent or less *C. atrorus* specific allele frequency). Lower boundary represents the lower cline limit (80 percent or less *C. atrorus* allele frequency). In December and March of year 1 CHU08 was not sampled, thus cline width during these sampling periods may have been artificially contracted as in the remaining sample dates CHU08 consistently set the upper limit of the cline.

Table 22. Variation in cline width and selection coefficients across seasons and years for TPI-B4

Year 1

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
December	CHU09	CHU14	0.62	0.21
March	CHU09	CHU11	0.31	0.83
August	CHU08	CHU15	0.93	0.09

Year 2

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU15	0.93	0.09
March	CHU08	CHU15	0.93	0.09
August	CHU08	CHU13	0.72	0.15

Year 3

	Upper boundary	Lower boundary	Cline width (km)	Selection coefficient
January	CHU08	CHU15	0.93	0.09
March	CHU08	CHU11	0.52	0.30
August	CHU08	CHU11	0.52	0.30

APPENDIX I.

CYPRINODON COLLECTION FOR DECEMBER 2000

Appendix I. *Cyprinodon* collection for December 2000

Site #	Collection #	CK	RAG	TPI-B
CHU01	EC0071.01	BB	BB	BB
CHU01	EC0071.02	BB	BB	BB
CHU01	EC0071.03	BB	BB	BB
CHU01	EC0071.04	BB	BB	BB
CHU01	EC0071.05	BB	BB	BB
CHU01	EC0071.06	BB	BB	BB
CHU01	EC0071.07	BB	BB	BB
CHU01	EC0071.08	BB	BB	BB
CHU01	EC0071.09	BB	BB	BB
CHU01	EC0071.10	BB	BB	BB
CHU01	EC0071.11	BB	BB	BB
CHU01	EC0071.12	BB	BB	BB
CHU01	EC0071.13	BB	BB	BB
CHU01	EC0071.14	BB	BB	BB
CHU01	EC0071.15	BB	BB	BB
CHU01	EC0071.16	BB	BB	BB
CHU01	EC0071.17	BB	BB	BB
CHU01	EC0071.18	BB	BB	BB
CHU01	EC0071.19	BB	BB	BB
CHU01	EC0071.20	BB	BB	BB
CHU01	EC0071.21	BB	BB	BB
CHU01	EC0071.22	BB	BB	BB
CHU01	EC0071.23	BB	BB	BB
CHU01	EC0071.24	BB	BB	BB
CHU01	EC0071.25	BB	BB	BB
CHU01	EC0071.26	BB	BB	BB
CHU01	EC0071.27	BB	BB	BB
CHU01	EC0071.28	BB	BB	BB
CHU01	EC0071.29	BB	BB	BB
CHU01	EC0071.30	BB	BB	BB
CHU02	EC0057.01	BB	BB	BB
CHU02	EC0057.02	BB	BB	BB
CHU02	EC0057.03	BB	BB	BB
CHU02	EC0057.04	BB	BB	BB
CHU02	EC0057.05	BB	BB	BB
CHU02	EC0057.06	BB	BB	BB
CHU02	EC0057.07	BB	BB	BB
CHU02	EC0057.08	BB	BB	BB
CHU02	EC0057.09	BB	BB	BB
CHU02	EC0057.10	BB	BB	BB

CHU02	EC0057.11	BB	BB	BB
CHU02	EC0057.12	BB	BB	BB
CHU02	EC0057.13	BB	BB	BB
CHU02	EC0057.14	BB	BB	BB
CHU02	EC0057.15	BB	BB	BB
CHU02	EC0057.16	BB	BB	BB
CHU02	EC0057.17	??	BB	BB
CHU02	EC0057.18	BB	BB	BB
CHU02	EC0057.19	BB	BB	BB
CHU02	EC0057.20	BB	BB	BB
CHU02	EC0057.21	BB	BB	BB
CHU02	EC0057.22	BB	BB	BB
CHU02	EC0057.23	BB	BB	BB
CHU02	EC0057.24	BB	BB	BB
CHU02	EC0057.25	BB	BB	BB
CHU02	EC0057.26	BB	BB	BB
CHU02	EC0057.27	BB	BB	BB
CHU02	EC0057.28	BB	BB	BB
CHU02	EC0057.29	BB	BB	BB
CHU02	EC0057.30	BB	BB	BB
CHU04	EC0058.01	BB	BB	BB
CHU04	EC0058.02	BB	BB	BB
CHU04	EC0058.03	BB	BB	BB
CHU04	EC0058.04	BB	BB	BB
CHU04	EC0058.05	AA	AA	AB
CHU04	EC0058.06	BB	BB	BB
CHU04	EC0058.07	BB	BB	BB
CHU04	EC0058.08	AB	BB	BB
CHU04	EC0058.09	BB	BB	BB
CHU04	EC0058.10	BB	BB	BB
CHU04	EC0058.11	BB	BB	BB
CHU04	EC0058.12	BB	BB	BB
CHU04	EC0058.13	BB	BB	BB
CHU04	EC0058.14	BB	BB	BB
CHU04	EC0058.15	AB	AB	AB
CHU04	EC0058.16	BB	??	BB
CHU04	EC0058.17	BB	BB	BB
CHU04	EC0058.18	BB	BB	BB
CHU04	EC0058.19	AB	BB	BB
CHU04	EC0058.20	BB	BB	BB
CHU04	EC0058.21	BB	BB	BB
CHU04	EC0058.22	BB	BB	BB
CHU04	EC0058.23	BB	BB	BB

CHU04	EC0058.24	BB	BB	BB
CHU04	EC0058.25	BB	BB	BB
CHU04	EC0058.26	BB	BB	BB
CHU04	EC0058.27	BB	BB	BB
CHU04	EC0058.28	BB	BB	BB
CHU04	EC0058.29	BB	BB	BB
CHU04	EC0058.30	BB	AB	BB
CHU05	EC0059.01	BB	BB	AB
CHU05	EC0059.02	AB	AA	AB
CHU05	EC0059.03	AB	AB	AB
CHU05	EC0059.04	AB	BB	BB
CHU05	EC0059.05	BB	BB	BB
CHU05	EC0059.06	BB	BB	BB
CHU05	EC0059.07	AA	AB	AB
CHU05	EC0059.08	AA	AA	AA
CHU05	EC0059.09	AB	AB	AB
CHU05	EC0059.10	BB	BB	BB
CHU05	EC0059.11	BB	BB	BB
CHU05	EC0059.12	BB	BB	BB
CHU05	EC0059.13	BB	BB	AB
CHU05	EC0059.14	AA	AA	AA
CHU05	EC0059.15	AA	AB	AA
CHU05	EC0059.16	AB	AB	AB
CHU05	EC0059.17	AA	BB	AB
CHU05	EC0059.18	BB	BB	BB
CHU05	EC0059.19	BB	BB	BB
CHU05	EC0059.20	BB	BB	BB
CHU05	EC0059.21	BB	AB	AB
CHU05	EC0059.22	AB	AB	AB
CHU05	EC0059.23	AB	AB	AB
CHU05	EC0059.24	AB	AB	BB
CHU05	EC0059.25	AB	BB	BB
CHU05	EC0059.26	AB	AB	AB
CHU05	EC0059.27	BB	BB	BB
CHU05	EC0059.28	AB	AB	AB
CHU05	EC0059.29	BB	BB	AB
CHU05	EC0059.30	BB	BB	AB
CHU06	EC0060.01	AA	AA	AA
CHU06	EC0060.02	AA	AA	AA
CHU06	EC0060.03	AA	AB	AA
CHU06	EC0060.04	AA	AA	AA

CHU06	EC0060.05	AA	AA	AA
CHU06	EC0060.06	AA	AA	AA
CHU06	EC0060.07	AA	AA	AA
CHU06	EC0060.08	AA	AA	AA
CHU06	EC0060.09	AA	AA	AA
CHU06	EC0060.10	AA	AA	AA
CHU06	EC0060.11	AA	AB	AA
CHU06	EC0060.12	AA	AA	AA
CHU06	EC0060.13	AA	AA	AA
CHU06	EC0060.14	AA	AA	AA
CHU06	EC0060.15	AA	AA	AA
CHU06	EC0060.16	AA	AA	AA
CHU06	EC0060.17	AA	AA	AA
CHU06	EC0060.18	AA	AA	AA
CHU06	EC0060.19	AA	AA	AA
CHU06	EC0060.20	AA	AA	AA
CHU06	EC0060.21	AA	AA	AA
CHU06	EC0060.22	AA	AA	AA
CHU06	EC0060.23	AA	AA	AA
CHU06	EC0060.24	AA	AA	AA
CHU06	EC0060.25	AA	AA	AA
CHU06	EC0060.26	AA	AA	AA
CHU06	EC0060.27	AA	AA	AA
CHU06	EC0060.28	AA	AA	AA
CHU06	EC0060.29	AA	AB	AA
CHU06	EC0060.30	AA	AA	AA

CHU09	EC0061.01	AB	BB	AB
CHU09	EC0061.02	AA	AA	AA
CHU09	EC0061.03	AB	BB	AB
CHU09	EC0061.04	BB	BB	BB
CHU09	EC0061.05	BB	BB	BB
CHU09	EC0061.06	BB	AB	AB
CHU09	EC0061.07	BB	BB	AB
CHU09	EC0061.08	BB	BB	AB
CHU09	EC0061.09	AB	AB	BB
CHU09	EC0061.10	AB	BB	AB
CHU09	EC0061.11	AB	AB	AB
CHU09	EC0061.12	AB	AB	AB
CHU09	EC0061.13	BB	BB	BB
CHU09	EC0061.14	AA	BB	AB
CHU09	EC0061.15	AB	AB	BB
CHU09	EC0061.16	BB	BB	AB
CHU09	EC0061.17	AB	BB	AB

CHU09	EC0061.18	BB	AB	AB
CHU09	EC0061.19	BB	BB	BB
CHU09	EC0061.20	AB	AB	AB
CHU09	EC0061.21	AA	AB	AA
CHU09	EC0061.22	BB	AA	AB
CHU09	EC0061.23	BB	BB	BB
CHU09	EC0061.24	AA	AA	AA
CHU09	EC0061.25	AA	BB	BB
CHU09	EC0061.26	BB	AB	BB
CHU09	EC0061.27	AB	BB	BB
CHU09	EC0061.28	AB	AA	AB
CHU09	EC0061.29	AB	BB	BB
CHU09	EC0061.30	NA	NA	NA
CHU10	EC0063.01	AA	AA	AA
CHU10	EC0063.02	AA	AB	AA
CHU10	EC0063.03	AA	AA	AB
CHU10	EC0063.04	AB	AB	AB
CHU10	EC0063.05	AB	AB	AA
CHU10	EC0063.06	AB	AB	AB
CHU10	EC0063.07	BB	BB	BB
CHU10	EC0063.08	AA	AB	AB
CHU10	EC0063.09	AB	AB	AB
CHU10	EC0063.10	AB	AA	AA
CHU10	EC0063.11	BB	AB	AB
CHU10	EC0063.12	BB	AB	BB
CHU10	EC0063.13	AA	AB	AB
CHU10	EC0063.14	AA	AA	AA
CHU10	EC0063.15	AA	AB	AB
CHU10	EC0063.16	AB	AB	BB
CHU10	EC0063.17	AA	AB	AB
CHU10	EC0063.18	AA	AA	AA
CHU10	EC0063.19	BB	BB	BB
CHU10	EC0063.20	BB	BB	AB
CHU10	EC0063.21	AA	AA	AA
CHU10	EC0063.22	AA	AA	AA
CHU10	EC0063.23	AB	AB	AB
CHU10	EC0063.24	AA	AB	AB
CHU10	EC0063.25	AA	BB	BB
CHU10	EC0063.26	AB	AA	AA
CHU10	EC0063.27	AB	AB	AB
CHU10	EC0063.28	AB	BB	AB
CHU10	EC0063.29	AB	AA	AB
CHU10	EC0063.30	AA	AB	AB

CHU11	EC0064.01	AB	BB	BB
CHU11	EC0064.02	AB	BB	BB
CHU11	EC0064.03	AB	BB	BB
CHU11	EC0064.04	AB	BB	BB
CHU11	EC0064.05	AA	BB	BB
CHU11	EC0064.06	AB	AB	AB
CHU11	EC0064.07	AB	BB	BB
CHU11	EC0064.08	AB	AB	AB
CHU11	EC0064.09	AB	BB	BB
CHU11	EC0064.10	BB	BB	BB
CHU11	EC0064.11	BB	AB	AB
CHU11	EC0064.12	AA	AA	AA
CHU11	EC0064.13	AA	AA	AB
CHU11	EC0064.14	AB	AB	AB
CHU11	EC0064.15	AB	BB	BB
CHU11	EC0064.16	AB	BB	AB
CHU11	EC0064.17	BB	BB	AB
CHU11	EC0064.18	AB	BB	AB
CHU11	EC0064.19	BB	BB	BB
CHU11	EC0064.20	AB	AB	AB
CHU11	EC0064.21	AB	AB	BB
CHU11	EC0064.22	AA	AA	AA
CHU11	EC0064.23	AA	AA	AA
CHU11	EC0064.24	BB	BB	BB
CHU11	EC0064.25	AB	BB	BB
CHU11	EC0064.26	AB	BB	AB
CHU11	EC0064.27	AB	BB	BB
CHU11	EC0064.28	AB	AB	AB
CHU11	EC0064.29	AA	AB	BB
CHU11	EC0064.30	AB	AA	AB

CHU12	EC0065.01	AA	AA	AA
CHU12	EC0065.02	AA	AA	AA
CHU12	EC0065.03	AA	AA	AA
CHU12	EC0065.04	AA	AA	AA
CHU12	EC0065.05	AA	AA	AA
CHU12	EC0065.06	AA	AA	AB
CHU12	EC0065.07	AA	AA	AA
CHU12	EC0065.08	AA	AB	AA
CHU12	EC0065.09	AA	AA	AA
CHU12	EC0065.10	AA	AA	AA
CHU12	EC0065.11	AB	AA	AA

CHU12	EC0065.12	AA	AB	AA
CHU12	EC0065.13	AA	AA	AA
CHU12	EC0065.14	AA	AB	AA
CHU12	EC0065.15	AA	AA	AA
CHU12	EC0065.16	AA	AA	AB
CHU12	EC0065.17	AA	AA	AA
CHU12	EC0065.18	AA	AA	AA
CHU12	EC0065.19	AA	??	AA
CHU12	EC0065.20	AA	??	AA
CHU12	EC0065.21	AB	AB	AB
CHU12	EC0065.22	AA	AB	AA
CHU12	EC0065.23	AA	AA	AA
CHU12	EC0065.24	AA	AA	AA
CHU12	EC0065.25	AA	BB	AB
CHU12	EC0065.26	AA	AA	AA
CHU12	EC0065.27	AA	AA	AA
CHU12	EC0065.28	AA	AA	AA
CHU12	EC0065.29	AA	AA	AA
CHU12	EC0065.30	AA	AA	AA

CHU13	EC0066.01	AB	AB	BB
CHU13	EC0066.02	BB	BB	AA
CHU13	EC0066.03	AB	BB	BB
CHU13	EC0066.04	AB	BB	AB
CHU13	EC0066.05	BB	BB	AB
CHU13	EC0066.06	AB	AA	AB
CHU13	EC0066.07	AB	BB	BB
CHU13	EC0066.08	AB	BB	AB
CHU13	EC0066.09	BB	BB	AB
CHU13	EC0066.10	AA	AA	AB
CHU13	EC0066.11	AB	BB	AB
CHU13	EC0066.12	AB	AA	AB
CHU13	EC0066.13	AB	AB	AA
CHU13	EC0066.14	AB	AB	AB
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CHU13	EC0066.16	AB	AB	AB
CHU13	EC0066.17	BB	BB	AB
CHU13	EC0066.18	AA	AB	BB
CHU13	EC0066.19	AA	BB	BB
CHU13	EC0066.20	BB	AB	BB
CHU13	EC0066.21	AB	BB	BB
CHU13	EC0066.22	AB	AB	AA
CHU13	EC0066.23	AB	AB	AB
CHU13	EC0066.24	AA	AB	AB

CHU13	EC0066.25	AA	BB	BB
CHU13	EC0066.26	AB	BB	AB
CHU13	EC0066.27	AA	AB	BB
CHU13	EC0066.28	??	AB	AB
CHU13	EC0066.29	AB	BB	BB
CHU13	EC0066.30	BB	BB	AB
CHU14	EC0067.01	AB	BB	BB
CHU14	EC0067.02	BB	BB	BB
CHU14	EC0067.03	AA	BB	AB
CHU14	EC0067.04	AB	BB	BB
CHU14	EC0067.05	BB	BB	BB
CHU14	EC0067.06	AB	BB	BB
CHU14	EC0067.07	AA	AB	AB
CHU14	EC0067.08	BB	BB	BB
CHU14	EC0067.09	BB	BB	AB
CHU14	EC0067.10	BB	BB	BB
CHU14	EC0067.11	AB	AB	AB
CHU14	EC0067.12	AB	AB	AB
CHU14	EC0067.13	AB	AB	BB
CHU14	EC0067.14	AB	BB	BB
CHU14	EC0067.15	AB	BB	AB
CHU14	EC0067.16	AA	AA	AA
CHU14	EC0067.17	AB	BB	AB
CHU14	EC0067.18	BB	BB	BB
CHU14	EC0067.19	AB	AA	AA
CHU14	EC0067.20	AB	AB	AB
CHU14	EC0067.21	BB	AA	AB
CHU14	EC0067.22	AA	AA	AB
CHU14	EC0067.23	AB	BB	BB
CHU14	EC0067.24	AB	AB	BB
CHU14	EC0067.25	BB	BB	AB
CHU14	EC0067.26	AB	AB	AB
CHU14	EC0067.27	BB	AB	AB
CHU14	EC0067.28	AB	AB	BB
CHU14	EC0067.29	BB	BB	AB
CHU14	EC0067.30	AA	AA	AB
CHU15	EC0068.01	AA	AA	AA
CHU15	EC0068.02	AA	AA	AA
CHU15	EC0068.03	AA	AA	AA
CHU15	EC0068.04	AA	AA	AA
CHU15	EC0068.05	AA	AA	AA

CHU15	EC0068.06	AA	AA	AA
CHU15	EC0068.07	AA	AA	AA
CHU15	EC0068.08	AB	AB	AB
CHU15	EC0068.09	AA	AA	AA
CHU15	EC0068.10	AA	AA	AB
CHU15	EC0068.11	AA	AA	AA
CHU15	EC0068.12	AA	AA	AB
CHU15	EC0068.13	AA	AA	AA
CHU15	EC0068.14	BB	AB	BB
CHU15	EC0068.15	AB	BB	BB
CHU15	EC0068.16	AA	AA	AA
CHU15	EC0068.17	AA	AA	AA
CHU15	EC0068.18	AA	AA	AA
CHU15	EC0068.19	AB	AB	AB
CHU15	EC0068.20	AB	AB	BB
CHU15	EC0068.21	AA	AA	AA
CHU15	EC0068.22	AA	AA	AA
CHU15	EC0068.23	AA	AA	AA
CHU15	EC0068.24	AA	AA	AA
CHU15	EC0068.25	AA	AA	AA
CHU15	EC0068.26	AB	AB	AB
CHU15	EC0068.27	AA	AA	AA
CHU15	EC0068.28	AA	AA	AA
CHU15	EC0068.29	AA	AA	AB
CHU15	EC0068.30	AB	AB	BB

CHU16	EC0069.01	AA	AA	AA
CHU16	EC0069.02	AA	AA	AA
CHU16	EC0069.03	AA	AA	AA
CHU16	EC0069.04	AA	AA	AA
CHU16	EC0069.05	AA	AA	AA
CHU16	EC0069.06	AA	AA	AA
CHU16	EC0069.07	AA	AA	AA
CHU16	EC0069.08	AA	??	AA
CHU16	EC0069.09	AA	AA	AA
CHU16	EC0069.10	AA	AA	AA
CHU16	EC0069.11	AA	AA	AA
CHU16	EC0069.12	AA	AA	AA
CHU16	EC0069.13	AA	AA	AB
CHU16	EC0069.14	AA	AA	AB
CHU16	EC0069.15	AA	AA	AA
CHU16	EC0069.16	AA	AA	AA
CHU16	EC0069.17	AA	AA	AA
CHU16	EC0069.18	AA	AA	AB

CHU16	EC0069.19	AA	AA	AA
CHU16	EC0069.20	AA	AA	AA
CHU16	EC0069.21	AA	AA	AB
CHU16	EC0069.22	AA	AA	AA
CHU16	EC0069.23	AA	AA	AB
CHU16	EC0069.24	AA	AA	AA
CHU16	EC0069.25	AA	AA	AA
CHU16	EC0069.26	AA	AA	AA
CHU16	EC0069.27	AA	AA	AA
CHU16	EC0069.28	AA	AA	AB
CHU16	EC0069.29	AA	AA	AA
CHU16	EC0069.30	AA	AA	AA
CHU17	EC0070.01	AA	AA	AA
CHU17	EC0070.02	AA	AA	AA
CHU17	EC0070.03	AA	AA	AB
CHU17	EC0070.04	??	AA	AA
CHU17	EC0070.05	AA	AA	AA
CHU17	EC0070.06	AA	AA	AA
CHU17	EC0070.07	AA	AA	AA
CHU17	EC0070.08	AA	AA	AA
CHU17	EC0070.09	AA	??	AA
CHU17	EC0070.10	??	AA	AA
CHU17	EC0070.11	AA	AA	AA
CHU17	EC0070.12	AA	AA	AA
CHU17	EC0070.13	AA	AA	AA
CHU17	EC0070.14	AA	AA	AA
CHU17	EC0070.15	AA	AA	AA
CHU17	EC0070.16	AA	AA	AA
CHU17	EC0070.17	AA	AA	AA
CHU17	EC0070.18	AA	AA	AA
CHU17	EC0070.19	AA	AA	AA
CHU17	EC0070.20	AA	AA	AA
CHU17	EC0070.21	AA	??	AA
CHU17	EC0070.22	AA	??	AA
CHU17	EC0070.23	AA	AA	AB
CHU17	EC0070.24	AA	AA	AA
CHU17	EC0070.25	AA	AA	AA
CHU17	EC0070.26	AA	AA	AA
CHU17	EC0070.27	AA	??	AA
CHU17	EC0070.28	??	AA	AA
CHU17	EC0070.29	AA	AA	AA
CHU17	EC0070.30	??	AA	AA

APPENDIX II.

CYPRINODON COLLECTION FOR MARCH 2001

Appendix II. *Cyprinodon* collection for March 2001

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0116.01	BB	BB	BB
CHU01	EC0116.02	BB	BB	BB
CHU01	EC0116.03	BB	BB	BB
CHU01	EC0116.04	BB	BB	BB
CHU01	EC0116.05	BB	BB	BB
CHU01	EC0116.06	BB	BB	BB
CHU01	EC0116.07	BB	BB	BB
CHU01	EC0116.08	BB	BB	BB
CHU01	EC0116.09	BB	BB	BB
CHU01	EC0116.10	BB	BB	BB
CHU01	EC0116.11	BB	BB	BB
CHU01	EC0116.12	BB	BB	BB
CHU01	EC0116.13	BB	BB	BB
CHU01	EC0116.14	BB	BB	BB
CHU01	EC0116.15	BB	BB	BB
CHU01	EC0116.16	BB	BB	BB
CHU01	EC0116.17	BB	BB	BB
CHU01	EC0116.18	BB	BB	BB
CHU01	EC0116.19	BB	BB	BB
CHU01	EC0116.20	BB	BB	BB
CHU01	EC0116.21	BB	BB	BB
CHU01	EC0116.22	BB	BB	BB
CHU01	EC0116.23	BB	BB	BB
CHU01	EC0116.24	BB	BB	BB
CHU01	EC0116.25	BB	BB	BB
CHU01	EC0116.26	BB	BB	BB
CHU01	EC0116.27	BB	BB	BB
CHU01	EC0116.28	BB	BB	BB
CHU01	EC0116.29	BB	BB	BB
CHU01	EC0116.30	BB	BB	BB
CHU02	EC0115.01	BB	BB	BB
CHU02	EC0115.02	BB	BB	BB
CHU02	EC0115.03	BB	BB	BB
CHU02	EC0115.04	BB	BB	BB
CHU02	EC0115.05	BB	BB	BB
CHU02	EC0115.06	BB	BB	BB
CHU02	EC0115.07	BB	BB	BB
CHU02	EC0115.08	BB	BB	BB

CHU02	EC0115.09	BB	BB	BB
CHU02	EC0115.10	BB	BB	BB
CHU02	EC0115.11	BB	BB	BB
CHU02	EC0115.12	BB	BB	BB
CHU02	EC0115.13	BB	BB	BB
CHU02	EC0115.14	BB	BB	BB
CHU02	EC0115.15	BB	BB	BB
CHU02	EC0115.16	BB	BB	BB
CHU02	EC0115.17	BB	BB	BB
CHU02	EC0115.18	BB	BB	BB
CHU02	EC0115.19	BB	BB	BB
CHU02	EC0115.20	BB	BB	BB
CHU02	EC0115.21	BB	BB	BB
CHU02	EC0115.22	BB	BB	BB
CHU02	EC0115.23	BB	BB	BB
CHU02	EC0115.24	BB	BB	BB
CHU02	EC0115.25	BB	BB	BB
CHU02	EC0115.26	BB	BB	BB
CHU02	EC0115.27	BB	BB	BB
CHU02	EC0115.28	BB	BB	BB
CHU02	EC0115.29	BB	BB	BB
CHU02	EC0115.30	BB	BB	BB
CHU04	EC0112.01	BB	BB	BB
CHU04	EC0112.02	BB	BB	BB
CHU04	EC0112.03	BB	BB	BB
CHU04	EC0112.04	BB	BB	BB
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CHU04	EC0112.06	BB	BB	BB
CHU04	EC0112.07	AB	BB	BB
CHU04	EC0112.08	BB	BB	BB
CHU04	EC0112.09	AB	BB	AB
CHU04	EC0112.10	BB	BB	BB
CHU04	EC0112.11	BB	BB	BB
CHU04	EC0112.12	BB	BB	BB
CHU04	EC0112.13	BB	BB	BB
CHU04	EC0112.14	BB	BB	BB
CHU04	EC0112.15	BB	BB	BB
CHU04	EC0112.16	BB	BB	BB
CHU04	EC0112.17	BB	BB	BB
CHU04	EC0112.18	BB	BB	BB
CHU04	EC0112.19	BB	BB	BB
CHU04	EC0112.20	BB	BB	BB
CHU04	EC0112.21	BB	BB	BB

CHU04	EC0112.22	BB	BB	BB
CHU04	EC0112.23	AB	BB	BB
CHU04	EC0112.24	AB	AB	BB
CHU04	EC0112.25	BB	AB	BB
CHU04	EC0112.26	BB	AB	BB
CHU04	EC0112.27	AB	AB	AB
CHU04	EC0112.28	BB	BB	BB
CHU04	EC0112.29	BB	BB	BB
CHU04	EC0112.30	BB	BB	BB
CHU05	EC0113.01	BB	BB	BB
CHU05	EC0113.02	AB	AB	AB
CHU05	EC0113.03	AB	AB	AB
CHU05	EC0113.04	AB	AB	BB
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CHU05	EC0113.06	AB	AB	AB
CHU05	EC0113.07	BB	BB	BB
CHU05	EC0113.08	BB	BB	BB
CHU05	EC0113.09	BB	BB	BB
CHU05	EC0113.10	AB	AB	AB
CHU05	EC0113.11	AB	AB	BB
CHU05	EC0113.12	BB	BB	BB
CHU05	EC0113.13	AB	AB	AB
CHU05	EC0113.14	AB	AA	AB
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CHU05	EC0113.17	??	BB	BB
CHU05	EC0113.18	AB	AB	BB
CHU05	EC0113.19	BB	BB	BB
CHU05	EC0113.20	AA	AA	AA
CHU05	EC0113.21	BB	AB	BB
CHU05	EC0113.22	AA	AB	AB
CHU05	EC0113.23	BB	BB	AB
CHU05	EC0113.24	BB	BB	BB
CHU05	EC0113.25	BB	BB	BB
CHU05	EC0113.26	BB	BB	BB
CHU05	EC0113.27	BB	BB	BB
CHU05	EC0113.28	??	??	??
CHU05	EC0113.29	??	??	??
CHU05	EC0113.30	??	??	??
CHU06	EC0114.01	AA	AA	AA
CHU06	EC0114.02	AA	AA	AA
CHU06	EC0114.03	AA	AA	AA

CHU06	EC0114.04	AA	AA	AA
CHU06	EC0114.05	AA	AA	AA
CHU06	EC0114.06	AA	AA	AA
CHU06	EC0114.07	AA	AA	AA
CHU06	EC0114.08	AA	AA	AA
CHU06	EC0114.09	AA	AA	AA
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CHU06	EC0114.12	AA	AA	AA
CHU06	EC0114.13	AA	AA	AA
CHU06	EC0114.14	AA	AA	AA
CHU06	EC0114.15	AA	AA	AA
CHU06	EC0114.16	AA	AA	AA
CHU06	EC0114.17	AA	AA	AA
CHU06	EC0114.18	AA	AA	AA
CHU06	EC0114.19	AA	AA	AA
CHU06	EC0114.20	AA	AA	AA
CHU06	EC0114.21	AA	AA	AA
CHU06	EC0114.22	AA	AA	AA
CHU06	EC0114.23	AA	AA	AA
CHU06	EC0114.24	AA	AA	AA
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CHU06	EC0114.26	AA	AA	AA
CHU06	EC0114.27	AA	AA	AA
CHU06	EC0114.28	AA	AA	AA
CHU06	EC0114.29	AA	AA	AA
CHU06	EC0114.30	AA	AA	AA
CHU09	EC0111.01	AB	AB	AB
CHU09	EC0111.02	AA	AA	AB
CHU09	EC0111.03	AA	AB	AB
CHU09	EC0111.04	AB	AB	AB
CHU09	EC0111.05	AB	AB	AB
CHU09	EC0111.06	AB	AB	AB
CHU09	EC0111.07	BB	AB	BB
CHU09	EC0111.08	AB	AA	AA
CHU09	EC0111.09	AA	AA	AA
CHU09	EC0111.10	BB	BB	BB
CHU09	EC0111.11	AA	??	AB
CHU09	EC0111.12	BB	AB	AB
CHU09	EC0111.13	AB	AB	AB
CHU09	EC0111.14	AB	AB	AB
CHU09	EC0111.15	AA	AB	AA
CHU09	EC0111.16	AB	BB	BB

CHU09	EC0111.17	BB	AB	BB
CHU09	EC0111.18	BB	BB	BB
CHU09	EC0111.19	AB	AB	AB
CHU09	EC0111.20	AB	AA	AB
CHU09	EC0111.21	AA	AA	AB
CHU09	EC0111.22	AB	AA	AB
CHU09	EC0111.23	AB	AA	AA
CHU09	EC0111.24	BB	BB	AB
CHU09	EC0111.25	BB	BB	AB
CHU09	EC0111.26	AA	AB	AB
CHU09	EC0111.27	AA	BB	AB
CHU09	EC0111.28	BB	BB	BB
CHU09	EC0111.29	NA	NA	NA
CHU09	EC0111.30	NA	NA	NA
CHU10	EC0110.01	AA	BB	BB
CHU10	EC0110.02	BB	BB	BB
CHU10	EC0110.03	AB	AA	AB
CHU10	EC0110.04	AA	AA	AA
CHU10	EC0110.05	AB	BB	BB
CHU10	EC0110.06	AB	AB	AB
CHU10	EC0110.07	BB	AA	AB
CHU10	EC0110.08	AB	BB	AA
CHU10	EC0110.09	BB	BB	BB
CHU10	EC0110.10	AB	AA	BB
CHU10	EC0110.11	AB	AA	AB
CHU10	EC0110.12	AA	AB	BB
CHU10	EC0110.13	BB	BB	BB
CHU10	EC0110.14	AA	AA	AB
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CHU10	EC0110.16	AA	AA	AA
CHU10	EC0110.17	AB	BB	AB
CHU10	EC0110.18	AA	AA	AA
CHU10	EC0110.19	AA	AA	AA
CHU10	EC0110.20	AA	AA	AB
CHU10	EC0110.21	AA	AA	AA
CHU10	EC0110.22	AB	AB	AA
CHU10	EC0110.23	AA	BB	AB
CHU10	EC0110.24	AB	AA	AA
CHU10	EC0110.25	AB	BB	BB
CHU10	EC0110.26	AA	AA	AA
CHU10	EC0110.27	AA	BB	BB
CHU10	EC0110.28	AA	AA	AA
CHU10	EC0110.29	AB	AB	AB

CHU10	EC0110.30	AA	AA	AA
CHU11	EC0109.01	AB	AB	AB
CHU11	EC0109.02	BB	BB	AB
CHU11	EC0109.03	BB	AB	AB
CHU11	EC0109.04	BB	AB	BB
CHU11	EC0109.05	AB	AA	AA
CHU11	EC0109.06	BB	BB	AB
CHU11	EC0109.07	AA	AA	AA
CHU11	EC0109.08	AB	AB	AA
CHU11	EC0109.09	AB	AB	AA
CHU11	EC0109.10	??	AB	AB
CHU11	EC0109.11	AA	AA	AA
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CHU11	EC0109.13	AB	AB	AA
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CHU11	EC0109.15	AB	AA	AA
CHU11	EC0109.16	AA	BB	AB
CHU11	EC0109.17	AB	AB	AB
CHU11	EC0109.18	AA	AB	BB
CHU11	EC0109.19	AB*	AB	AB
CHU11	EC0109.20	AB	AB	BB
CHU11	EC0109.21	AB	AA	AB
CHU11	EC0109.22	BB	AB	BB
CHU11	EC0109.23	AA	AA	AB
CHU11	EC0109.24	AB	AB	AB
CHU11	EC0109.25	AA	AB	AB
CHU11	EC0109.26	AA	AB	AB
CHU11	EC0109.27	AB	AB	AB
CHU11	EC0109.28	AA	AB	AA
CHU11	EC0109.29	AB	AB	AB
CHU11	EC0109.30	AB	AB	AB
CHU12	EC0108.01	AA	AB	AA
CHU12	EC0108.02	AA	AA	AA
CHU12	EC0108.03	AA	AA	AA
CHU12	EC0108.04	AA	AA	AA
CHU12	EC0108.05	AB	AA	AB
CHU12	EC0108.06	AA	AA	AA
CHU12	EC0108.07	AA	AB	AB
CHU12	EC0108.08	AB	AB	AB
CHU12	EC0108.09	AB	AA	AB
CHU12	EC0108.10	AB	AA	AB
CHU12	EC0108.11	AA	AB	AB

CHU12	EC0108.12	AA	AA	AA
CHU12	EC0108.13	AA	AB	AA
CHU12	EC0108.14	AA	AA	AA
CHU12	EC0108.15	AA	AA	AA
CHU12	EC0108.16	AA	AA	AA
CHU12	EC0108.17	AA	AA	AA
CHU12	EC0108.18	AA	AB	AA
CHU12	EC0108.19	AA	AA	AA
CHU12	EC0108.20	AA	AA	AA
CHU12	EC0108.21	AA	AB	AA
CHU12	EC0108.22	AA	AA	AA
CHU12	EC0108.23	AA	AA	AA
CHU12	EC0108.24	AA	AA	AA
CHU12	EC0108.25	AA	AA	AA
CHU12	EC0108.26	AA	AB	AB
CHU12	EC0108.27	AA	AA	AA
CHU12	EC0108.28	AA	AA	AA
CHU12	EC0108.29	AA	AA	AA
CHU12	EC0108.30	AA	AA	AA
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CHU13	EC0107.03	AB	AB	AA
CHU13	EC0107.04	AB	AB	AA
CHU13	EC0107.05	AB	AB	AA
CHU13	EC0107.06	AA	BB	AA
CHU13	EC0107.07	BB	BB	AA
CHU13	EC0107.08	AB	BB	AA
CHU13	EC0107.09	AA	AA	AA
CHU13	EC0107.10	AA	AA	AA
CHU13	EC0107.11	AA	AA	AA
CHU13	EC0107.12	AB	AB	AA
CHU13	EC0107.13	AB	AB	AA
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CHU13	EC0107.17	AA	AA	AA
CHU13	EC0107.18	AB	AB	AB
CHU13	EC0107.19	??	AA	AB
CHU13	EC0107.20	AA	AA	AB
CHU13	EC0107.21	AA	AA	AA
CHU13	EC0107.22	AA	AA	AB
CHU13	EC0107.23	AA	AA	AB
CHU13	EC0107.24	AB	AB	AB

CHU13	EC0107.25	BB	BB	AA
CHU13	EC0107.26	AB	AB	AA
CHU13	EC0107.27	BB	AB	AA
CHU13	EC0107.28	AA	AA	AA
CHU13	EC0107.29	AB	AB	AB
CHU13	EC0107.30	AB	AB	BB
CHU14	EC0106.01	AA	AA	AB
CHU14	EC0106.02	AB	AA	AA
CHU14	EC0106.03	AA	AA	AA
CHU14	EC0106.04	AA	AA	AB
CHU14	EC0106.05	??	AA	AA
CHU14	EC0106.06	AA	AA	AA
CHU14	EC0106.07	AA	AA	AA
CHU14	EC0106.08	AB	AA	AA
CHU14	EC0106.09	AA	AA	AA
CHU14	EC0106.10	AA	AA	AA
CHU14	EC0106.11	AA	AA	AA
CHU14	EC0106.12	AA	AA	AA
CHU14	EC0106.13	AA	AA	AA
CHU14	EC0106.14	AA	AA	??
CHU14	EC0106.15	AA	AA	AA
CHU14	EC0106.16	AA	AA	AA
CHU14	EC0106.17	AA	AA	AA
CHU14	EC0106.18	AA	AA	??
CHU14	EC0106.19	??	AA	AA
CHU14	EC0106.20	??	AA	AA
CHU14	EC0106.21	AA	AA	AA
CHU14	EC0106.22	??	AA	AA
CHU14	EC0106.23	AA	AA	AA
CHU14	EC0106.24	??	AA	AA
CHU14	EC0106.25	AA	AA	AA
CHU14	EC0106.26	AA	AA	AA
CHU14	EC0106.27	??	AA	AA
CHU14	EC0106.28	AA	AA	AA
CHU14	EC0106.29	??	AA	??
CHU14	EC0106.30	AA	AA	AA
CHU15	EC0105.01	AA	AB	BB
CHU15	EC0105.02	AA	??	AA
CHU15	EC0105.03	AA	AA	AA
CHU15	EC0105.04	AA	AA	AA
CHU15	EC0105.05	??	AA	AA

CHU15	EC0105.06	AA	AA	AA
CHU15	EC0105.07	AA	AA	AA
CHU15	EC0105.08	AA	AA	AA
CHU15	EC0105.09	??	AA	AA
CHU15	EC0105.10	??	??	??
CHU15	EC0105.11	AA	AA	AA
CHU15	EC0105.12	AA	AA	AA
CHU15	EC0105.13	AA	AA	AA
CHU15	EC0105.14	AA	BB	BB
CHU15	EC0105.15	AA	AB	BB
CHU15	EC0105.16	AA	AA	AA
CHU15	EC0105.17	AA	AA	AA
CHU15	EC0105.18	AA	AA	AA
CHU15	EC0105.19	AA	AA	AA
CHU15	EC0105.20	AA	AB	AA
CHU15	EC0105.21	AB	BB	AB
CHU15	EC0105.22	AA	AB	AA
CHU15	EC0105.23	AA	AA	AA
CHU15	EC0105.24	AA	AA	AA
CHU15	EC0105.25	AA	AB	AA
CHU15	EC0105.26	AA	AA	AA
CHU15	EC0105.27	AB	AA	AB
CHU15	EC0105.28	AA	AA	AB
CHU15	EC0105.29	BB	BB	BB
CHU15	EC0105.30	AA	AA	AA
CHU16	EC0104.01	AA	AA	AA
CHU16	EC0104.02	AA	AA	AA
CHU16	EC0104.03	AA	AA	AA
CHU16	EC0104.04	AA	AA	AA
CHU16	EC0104.05	AA	AA	AA
CHU16	EC0104.06	AA	AA	AA
CHU16	EC0104.07	AA	AA	AA
CHU16	EC0104.08	AA	AA	AA
CHU16	EC0104.09	AA	AA	AA
CHU16	EC0104.10	AA	AA	AA
CHU16	EC0104.11	AA	AA	AB
CHU16	EC0104.12	AA	AA	AA
CHU16	EC0104.13	AA	AA	AA
CHU16	EC0104.14	AA	AA	AA
CHU16	EC0104.15	AA	AA	AA
CHU16	EC0104.16	AA	AA	AA
CHU16	EC0104.17	AA	AA	AA
CHU16	EC0104.18	AA	AA	AA

CHU16	EC0104.19	AA	AA	AA
CHU16	EC0104.20	AA	AA	AA
CHU16	EC0104.21	AA	AA	AB
CHU16	EC0104.22	AA	AA	AA
CHU16	EC0104.23	AA	AA	AA
CHU16	EC0104.24	AA	AA	AA
CHU16	EC0104.25	??	AA	??
CHU16	EC0104.26	AA	AA	AB
CHU16	EC0104.27	AA	AA	AA
CHU16	EC0104.28	AA	AA	AA
CHU16	EC0104.29	??	AA	AB
CHU16	EC0104.30	AA	AA	AA
CHU17	EC0103.01	??	AA	AA
CHU17	EC0103.02	??	??	??
CHU17	EC0103.03	??	AA	AA
CHU17	EC0103.04	??	??	??
CHU17	EC0103.05	??	??	??
CHU17	EC0103.06	??	AA	AA
CHU17	EC0103.07	AA	AA	AA
CHU17	EC0103.08	AA	AA	AA
CHU17	EC0103.09	AA	AA	AB
CHU17	EC0103.10	AA	AA	AA
CHU17	EC0103.11	AA	AA	AA
CHU17	EC0103.12	AA	AA	AA
CHU17	EC0103.13	AA	AA	AA
CHU17	EC0103.14	AA	AA	AA
CHU17	EC0103.15	AA	AA	AA
CHU17	EC0103.16	AA	AA	AA
CHU17	EC0103.17	AA	AA	AB
CHU17	EC0103.18	AA	AA	AA
CHU17	EC0103.19	??	??	??
CHU17	EC0103.20	AA	AA	AA
CHU17	EC0103.21	AA	AA	AA
CHU17	EC0103.22	AA	AA	AA
CHU17	EC0103.23	AA	AA	AA
CHU17	EC0103.24	AA	AA	AA
CHU17	EC0103.25	AA	AA	AA
CHU17	EC0103.26	AA	AA	AA
CHU17	EC0103.27	AA	AA	AB
CHU17	EC0103.28	AA	AA	AA
CHU17	EC0103.29	AA	AA	AA
CHU17	EC0103.30	AA	AA	AA

APPENDIX III.

CYPRINODON COLLECTION FOR AUGUST 2001

Appendix III. *Cyprinodon* collection for August 2001

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0151.01	BB	BB	BB
CHU01	EC0151.02	BB	BB	BB
CHU01	EC0151.03	BB	BB	BB
CHU01	EC0151.04	BB	BB	BB
CHU01	EC0151.05	BB	BB	BB
CHU01	EC0151.06	BB	BB	BB
CHU01	EC0151.07	BB	??	BB
CHU01	EC0151.08	BB	??	BB
CHU01	EC0151.09	BB	??	BB
CHU01	EC0151.10	BB	BB	BB
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CHU17	EC0138.10	AA	AA	AA
CHU17	EC0138.11	AA	AA	AA
CHU17	EC0138.12	AA	AA	AA
CHU17	EC0138.13	AA	AA	AA
CHU17	EC0138.14	AA	AA	AA
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CHU17	EC0138.17	AA	AA	AA
CHU17	EC0138.18	AA	AA	AA
CHU17	EC0138.19	AA	AA	AA
CHU17	EC0138.20	AA	AA	AA
CHU17	EC0138.21	AA	AA	AA
CHU17	EC0138.22	AA	AA	AA
CHU17	EC0138.23	AA	AA	AA
CHU17	EC0138.24	AA	AA	AA
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CHU17	EC0138.26	AA	AA	AA
CHU17	EC0138.27	AA	AA	AA
CHU17	EC0138.28	AA	AA	AA
CHU17	EC0138.29	AA	AA	AA
CHU17	EC0138.30	AA	AA	AA

APPENDIX IV.

CYPRINODON COLLECTION FOR JANUARY 2002

Appendix IV. *Cyprinodon* collection for January 2002

Site #	Collect #	CK	RAG	TPI-B	
CHU01		EC0187.01	BB	BB	BB
CHU01		EC0187.02	BB	BB	BB
CHU01		EC0187.03	BB	BB	BB
CHU01		EC0187.04	BB	BB	BB
CHU01		EC0187.05	BB	BB	BB
CHU01		EC0187.06	BB	BB	BB
CHU01		EC0187.07	BB	BB	BB
CHU01		EC0187.08	BB	BB	BB
CHU01		EC0187.09	BB	BB	BB
CHU01		EC0187.10	BB	BB	BB
CHU01		EC0187.11	BB	BB	BB
CHU01		EC0187.12	BB	BB	BB
CHU01		EC0187.13	BB	BB	BB
CHU01		EC0187.14	BB	BB	BB
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CHU01		EC0187.17	BB	BB	BB
CHU01		EC0187.18	BB	BB	BB
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CHU01		EC0187.27	BB	BB	BB
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CHU01		EC0187.29	BB	BB	BB
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CHU02		EC0186.07	BB	BB	BB
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CHU05	EC0183.21	BB	BB	BB
CHU05	EC0183.22	BB	BB	BB
CHU05	EC0183.23	AB	AB	AB
CHU05	EC0183.24	AB	AB	AB
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CHU05	EC0183.26	BB	BB	BB
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CHU05	EC0183.28	AB	BB	BB
CHU05	EC0183.29	AB	BB	AB
CHU05	EC0183.30	BB	BB	BB
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CHU06	EC0182.02	AA	AA	AA
CHU06	EC0182.03	AA	AA	AA
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CHU06	EC0182.07	AA	AA	AA
CHU06	EC0182.08	AA	AA	AA
CHU06	EC0182.09	AA	AA	AA
CHU06	EC0182.10	AA	AA	AA
CHU06	EC0182.11	AA	AA	AA
CHU06	EC0182.12	AA	AA	AA
CHU06	EC0182.13	AA	AA	AA
CHU06	EC0182.14	AA	AA	AA
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CHU06	EC0182.17	AA	AA	AA
CHU06	EC0182.18	AA	AA	AA
CHU06	EC0182.19	AA	AA	AA
CHU06	EC0182.20	AA	AA	AA

CHU06	EC0182.21	AA	AA	AA
CHU06	EC0182.22	AA	AA	AA
CHU06	EC0182.23	AB	AA	AA
CHU06	EC0182.24	AA	AA	AA
CHU06	EC0182.25	AA	AA	AA
CHU06	EC0182.26	AA	AA	AA
CHU06	EC0182.27	AA	AA	AA
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CHU07	EC0181.07	AA	AA	AA
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CHU07	EC0181.12	AA	AA	AA
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CHU07	EC0181.17	AA	AA	AA
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CHU07	EC0181.19	AA	AA	AA
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CHU07	EC0181.21	AA	AA	AA
CHU07	EC0181.22	AA	AA	AA
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CHU07	EC0181.27	AA	AA	AA
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CHU08	EC0180.07	BB	AB	AB
CHU08	EC0180.08	BB	BB	AB
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CHU08	EC0180.27	AB	BB	BB
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CHU09	EC0179.08	AB	AB	BB
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CHU09	EC0179.16	AB	AB	AB
CHU09	EC0179.17	AB	BB	BB
CHU09	EC0179.18	AB	??	BB
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CHU09	EC0179.20	BB	BB	BB
CHU09	EC0179.21	BB	BB	BB
CHU09	EC0179.22	BB	BB	BB
CHU09	EC0179.23	AB	BB	AB
CHU09	EC0179.24	BB	BB	BB
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CHU10	EC0178.07	AB	AB	AB
CHU10	EC0178.08	AA	AB	AA
CHU10	EC0178.09	AB	AB	AB
CHU10	EC0178.10	BB	AB	BB
CHU10	EC0178.11	AB	AB	AB
CHU10	EC0178.12	AB	BB	BB
CHU10	EC0178.13	AA	AA	AA
CHU10	EC0178.14	AB	AA	AA
CHU10	EC0178.15	BB	BB	BB
CHU10	EC0178.16	BB	BB	BB
CHU10	EC0178.17	BB	BB	BB
CHU10	EC0178.18	AB	BB	AB
CHU10	EC0178.19	AA	AA	AA
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CHU10	EC0178.22	AB	BB	AB
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CHU10	EC0178.24	AB	BB	BB
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CHU10	EC0178.26	AB	AA	AB
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CHU10	EC0178.28	AB	AA	AA

CHU10	EC0178.29	AA	AA	AA
CHU10	EC0178.30	AA	AA	AA
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CHU11	EC0177.07	AB	AB	AB
CHU11	EC0177.08	AB	BB	AB
CHU11	EC0177.09	BB	AB	BB
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CHU11	EC0177.12	AA	BB	AB
CHU11	EC0177.13	AB	AB	AB
CHU11	EC0177.14	AB	AB	AB
CHU11	EC0177.15	AA	AA	AA
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CHU11	EC0177.17	AA	AB	AB
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CHU11	EC0177.19	AB	AB	BB
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CHU11	EC0177.23	AB	AB	AB
CHU11	EC0177.24	BB	BB	BB
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CHU12	EC0176.17	AA	AB	AA
CHU12	EC0176.18	AA	AA	AA
CHU12	EC0176.19	AA	AA	AA
CHU12	EC0176.20	AA	AA	AB
CHU12	EC0176.21	AA	AB	AA
CHU12	EC0176.22	AA	AA	AA
CHU12	EC0176.23	AA	AA	AA
CHU12	EC0176.24	AA	AA	AA
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CHU12	EC0176.30	AA	AA	AB

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CHU13	EC0175.07	BB	AB	AB
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CHU13	EC0175.10	AB	AB	BB
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CHU14	EC0174.07	AA	AA	AA
CHU14	EC0174.08	BB	BB	AB
CHU14	EC0174.09	AA	AA	AA
CHU14	EC0174.10	AB	BB	AB
CHU14	EC0174.11	AB	AB	AB
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CHU14	EC0174.14	AA	AA	AA
CHU14	EC0174.15	BB	BB	BB
CHU14	EC0174.16	AA	AA	AA
CHU14	EC0174.17	AA	BB	AB
CHU14	EC0174.18	AA	AA	AA
CHU14	EC0174.19	AA	AA	AA
CHU14	EC0174.20	AB	BB	AB
CHU14	EC0174.21	BB	AB	AB
CHU14	EC0174.22	BB	BB	BB
CHU14	EC0174.23	AB	AB	AB
CHU14	EC0174.24	AB	BB	AB
CHU14	EC0174.25	AB	BB	AB
CHU14	EC0174.26	AB	AB	AB
CHU14	EC0174.27	AA	AA	AA
CHU14	EC0174.28	AA	AA	AA
CHU14	EC0174.29	AB	AB	AB
CHU14	EC0174.30	AA	AA	AB
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CHU15	EC0173.02	AA	AA	AA
CHU15	EC0173.03	AA	AA	AA

CHU15	EC0173.04	AA	AA	AA
CHU15	EC0173.05	AA	AA	AA
CHU15	EC0173.06	AA	AA	AA
CHU15	EC0173.07	AA	AA	AB
CHU15	EC0173.08	AB	AA	BB
CHU15	EC0173.09	AB	BB	BB
CHU15	EC0173.10	AA	AB	AB
CHU15	EC0173.11	AA	AA	AB
CHU15	EC0173.12	AA	AA	AA
CHU15	EC0173.13	AA	AA	AB
CHU15	EC0173.14	AA	AA	AA
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CHU15	EC0173.17	AA	AB	AB
CHU15	EC0173.18	AA	AA	AB
CHU15	EC0173.19	AA	AA	AB
CHU15	EC0173.20	AA	AA	AA
CHU15	EC0173.21	AA	AA	AA
CHU15	EC0173.22	AA	AB	AB
CHU15	EC0173.23	AA	AA	AA
CHU15	EC0173.24	BB	BB	AB
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CHU15	EC0173.27	AA	AA	AA
CHU15	EC0173.28	AA	AA	AA
CHU15	EC0173.29	AA	AA	AA
CHU15	EC0173.30	BB	BB	BB

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CHU16	EC0172.02	AA	AA	AA
CHU16	EC0172.03	AA	AA	AA
CHU16	EC0172.04	AA	AA	AA
CHU16	EC0172.05	AA	AA	AA
CHU16	EC0172.06	AA	AA	AB
CHU16	EC0172.07	AA	AA	AA
CHU16	EC0172.08	AA	AA	AA
CHU16	EC0172.09	AA	AA	AA
CHU16	EC0172.10	AA	AA	AA
CHU16	EC0172.11	AA	AA	AA
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CHU16	EC0172.13	AA	AA	AA
CHU16	EC0172.14	AA	AA	AA
CHU16	EC0172.15	AA	AA	AA
CHU16	EC0172.16	AA	AA	AA

CHU16	EC0172.17	AA	AA	AA
CHU16	EC0172.18	AA	AA	AA
CHU16	EC0172.19	AA	AA	AA
CHU16	EC0172.20	AA	AA	AB
CHU16	EC0172.21	AA	AA	AA
CHU16	EC0172.22	AA	AA	AA
CHU16	EC0172.23	AA	AA	AA
CHU16	EC0172.24	AA	AA	AA
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CHU16	EC0172.27	AA	AA	AA
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CHU16	EC0172.29	AA	AA	AB
CHU16	EC0172.30	AA	AA	AA
CHU17	EC0171.01	AA	AA	AA
CHU17	EC0171.02	AA	AA	AA
CHU17	EC0171.03	AA	AA	AA
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CHU17	EC0171.05	AA	AA	AA
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CHU17	EC0171.11	AA	AA	AB
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CHU17	EC0171.17	AA	AA	AA
CHU17	EC0171.18	AA	AA	AB
CHU17	EC0171.19	AA	AA	AA
CHU17	EC0171.20	AA	AA	AA
CHU17	EC0171.21	AA	AA	AB
CHU17	EC0171.22	AA	AA	AA
CHU17	EC0171.23	AA	AA	AB
CHU17	EC0171.24	AA	AA	AA
CHU17	EC0171.25	AA	AA	AA
CHU17	EC0171.26	AA	AA	AA
CHU17	EC0171.27	AA	AA	AA
CHU17	EC0171.28	AA	AA	AA
CHU17	EC0171.29	AA	AA	AA

CHU17	EC0171.30	AA	AA	AA

APPENDIX V.

CYPRINODON COLLECTION FOR MARCH 2002

Appendix V. *Cyprinodon* collection for March 2002

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0217.01	BB	BB	BB
CHU01	EC0217.02	BB	BB	BB
CHU01	EC0217.03	BB	BB	BB
CHU01	EC0217.04	BB	BB	BB
CHU01	EC0217.05	BB	BB	BB
CHU01	EC0217.06	BB	BB	BB
CHU01	EC0217.07	BB	BB	BB
CHU01	EC0217.08	BB	BB	BB
CHU01	EC0217.09	BB	BB	BB
CHU01	EC0217.10	BB	BB	BB
CHU01	EC0217.11	??	BB	??
CHU01	EC0217.12	BB	BB	BB
CHU01	EC0217.13	BB	BB	BB
CHU01	EC0217.14	BB	BB	BB
CHU01	EC0217.15	BB	??	BB
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CHU01	EC0217.17	BB	BB	BB
CHU01	EC0217.18	BB	BB	BB
CHU01	EC0217.19	BB	BB	BB
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CHU01	EC0217.21	BB	BB	BB
CHU01	EC0217.22	BB	BB	BB
CHU01	EC0217.23	BB	BB	BB
CHU01	EC0217.24	BB	BB	BB
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CHU01	EC0217.26	BB	BB	BB
CHU01	EC0217.27	BB	BB	BB
CHU01	EC0217.28	BB	BB	BB
CHU01	EC0217.29	NA	NA	NA
CHU01	EC0217.30	NA	NA	NA
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CHU02	EC0216.03	BB	BB	BB
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CHU02	EC0216.06	BB	BB	BB
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CHU02	EC0216.08	BB	BB	BB
CHU02	EC0216.09	BB	BB	BB

CHU02	EC0216.10	BB	??	BB
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CHU03	EC0215.22	BB	BB	BB

CHU03	EC0215.23	BB	BB	BB
CHU03	EC0215.24	AB	BB	BB
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CHU04	EC0214.09	BB	AB	BB
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CHU04	EC0214.11	BB	BB	BB
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CHU04	EC0214.13	AB	BB	BB
CHU04	EC0214.14	BB	BB	BB
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CHU04	EC0214.17	BB	BB	BB
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CHU05	EC0213.07	AA	AA	AA
CHU05	EC0213.08	BB	BB	BB
CHU05	EC0213.09	BB	BB	BB
CHU05	EC0213.10	BB	BB	BB
CHU05	EC0213.11	AA	AA	AA
CHU05	EC0213.12	AB	AA	AA
CHU05	EC0213.13	AA	AA	AA
CHU05	EC0213.14	AA	AA	AA
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CHU05	EC0213.21	BB	??	BB
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CHU05	EC0213.24	BB	BB	BB
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CHU05	EC0213.28	BB	BB	BB
CHU05	EC0213.29	BB	??	BB
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CHU06	EC0212.02	AA	AA	AA
CHU06	EC0212.03	AA	AA	AA
CHU06	EC0212.04	AA	AA	AA
CHU06	EC0212.05	AB	AA	AA
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CHU06	EC0212.13	AA	AA	AA
CHU06	EC0212.14	AA	AA	AA
CHU06	EC0212.15	AA	AA	AA
CHU06	EC0212.16	AA	AA	AA

CHU06	EC0212.17	AB	AA	AA
CHU06	EC0212.18	AA	AA	AA
CHU06	EC0212.19	AA	AA	AA
CHU06	EC0212.20	AA	AA	AA
CHU06	EC0212.21	AA	AA	AA
CHU06	EC0212.22	AB	AA	AA
CHU06	EC0212.23	AA	AA	AA
CHU06	EC0212.24	AA	AB	AA
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CHU07	EC0211.17	AA	AA	AA
CHU07	EC0211.18	AA	AA	AA
CHU07	EC0211.19	AA	AA	AA
CHU07	EC0211.20	AA	AA	AA
CHU07	EC0211.21	AA	AA	AA
CHU07	EC0211.22	AA	AA	AA
CHU07	EC0211.23	AA	AA	AA
CHU07	EC0211.24	AA	AA	AA
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CHU07	EC0211.27	AA	AA	AA
CHU07	EC0211.28	AA	AA	AA
CHU07	EC0211.29	AA	AA	AA

CHU07	EC0211.30	AA	AA	AA
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CHU08	EC0210.02	AB	BB	BB
CHU08	EC0210.03	AB	AB	AB
CHU08	EC0210.04	AB	AB	AB
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CHU08	EC0210.07	BB	AB	AB
CHU08	EC0210.08	BB	AB	BB
CHU08	EC0210.09	BB	BB	BB
CHU08	EC0210.10	AB	BB	BB
CHU08	EC0210.11	AA	AA	AA
CHU08	EC0210.12	AA	BB	BB
CHU08	EC0210.13	AB	AA	AA
CHU08	EC0210.14	AA	BB	AB
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CHU08	EC0210.17	AB	AB	BB
CHU08	EC0210.18	AA	AB	BB
CHU08	EC0210.19	AA	AB	AB
CHU08	EC0210.20	AB	AB	BB
CHU08	EC0210.21	AB	BB	AB
CHU08	EC0210.22	BB	AB	AB
CHU08	EC0210.23	AA	AB	AB
CHU08	EC0210.24	AB	AB	??
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CHU08	EC0210.29	AB	AB	AB
CHU08	EC0210.30	AA	BB	AB
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CHU09	EC0209.04	AA	BB	BB
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CHU09	EC0209.06	AB	AB	AB
CHU09	EC0209.07	AB	BB	BB
CHU09	EC0209.08	AB	BB	BB
CHU09	EC0209.09	AB	AB	AB
CHU09	EC0209.10	BB	BB	BB

CHU09	EC0209.11	AA	AA	AA
CHU09	EC0209.12	AA	BB	AB
CHU09	EC0209.13	AB	BB	BB
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CHU09	EC0209.17	AB	AB	AB
CHU09	EC0209.18	BB	BB	AB
CHU09	EC0209.19	AB	BB	AB
CHU09	EC0209.20	BB	BB	BB
CHU09	EC0209.21	AA	AA	AA
CHU09	EC0209.22	AB	AB	AB
CHU09	EC0209.23	AA	AB	AB
CHU09	EC0209.24	AA	AA	AB
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CHU09	EC0209.27	AB	AB	AB
CHU09	EC0209.28	BB	AB	BB
CHU09	EC0209.29	AA	AA	AA
CHU09	EC0209.30	AB	BB	BB
CHU10	EC0208.01	AA	AB	AA
CHU10	EC0208.02	AB	BB	BB
CHU10	EC0208.03	AA	AA	AA
CHU10	EC0208.04	??	AA	AB
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CHU10	EC0208.14	BB	BB	BB
CHU10	EC0208.15	AA	AA	AA
CHU10	EC0208.16	AB	AB	??
CHU10	EC0208.17	AB	AB	AA
CHU10	EC0208.18	AA	AA	AA
CHU10	EC0208.19	AB	AB	AB
CHU10	EC0208.20	BB	AB	BB
CHU10	EC0208.21	AB	AB	AB
CHU10	EC0208.22	AA	AA	AA
CHU10	EC0208.23	AB	BB	BB

CHU10	EC0208.24	AB	AA	AB
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CHU11	EC0207.07	BB	BB	BB
CHU11	EC0207.08	AB	AB	AB
CHU11	EC0207.09	AB	AB	AB
CHU11	EC0207.10	AB	AB	AB
CHU11	EC0207.11	AB	AB	AB
CHU11	EC0207.12	BB	BB	AB
CHU11	EC0207.13	AB	BB	BB
CHU11	EC0207.14	AB	AB	BB
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CHU11	EC0207.17	??	AB	BB
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CHU11	EC0207.19	AB	BB	AA
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CHU11	EC0207.21	BB	BB	BB
CHU11	EC0207.22	AB	BB	AB
CHU11	EC0207.23	AB	AB	AB
CHU11	EC0207.24	BB	AB	AB
CHU11	EC0207.25	AB	AB	AB
CHU11	EC0207.26	AA	??	AB
CHU11	EC0207.27	BB	??	BB
CHU11	EC0207.28	AA	AA	AA
CHU11	EC0207.29	AA	AB	AB
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CHU12	EC0206.03	AA	AA	AA
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CHU12	EC0206.05	AA	AB	AA
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CHU12	EC0206.07	AA	AA	AA
CHU12	EC0206.08	AA	AA	AA
CHU12	EC0206.09	AA	AA	AA
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CHU12	EC0206.17	AA	AA	AA
CHU12	EC0206.18	AA	AA	AA
CHU12	EC0206.19	AA	AB	AB
CHU12	EC0206.20	AA	AA	AA
CHU12	EC0206.21	AA	AA	AA
CHU12	EC0206.22	AB	AA	AA
CHU12	EC0206.23	AA	AB	AA
CHU12	EC0206.24	AA	AB	AA
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CHU13	EC0205.12	AB	AB	AB
CHU13	EC0205.13	AB	AB	AB
CHU13	EC0205.14	AA	AA	AB
CHU13	EC0205.15	AB	AB	BB
CHU13	EC0205.16	AB	AB	BB
CHU13	EC0205.17	AA	AA	AA

CHU13	EC0205.18	AB	BB	BB
CHU13	EC0205.19	AA	AA	AA
CHU13	EC0205.20	AB	AB	AB
CHU13	EC0205.21	BB	AA	AB
CHU13	EC0205.22	AA	AA	AA
CHU13	EC0205.23	AA	AA	BB
CHU13	EC0205.24	AA	AA	AA
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CHU14	EC0204.03	AA	AA	AB
CHU14	EC0204.04	AA	AA	AA
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CHU14	EC0204.12	AA	AA	AA
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CHU14	EC0204.16	BB	AB	AB
CHU14	EC0204.17	AA	AA	AA
CHU14	EC0204.18	AA	AA	AA
CHU14	EC0204.19	AB	BB	AB
CHU14	EC0204.20	AA	AA	AA
CHU14	EC0204.21	AA	AA	AA
CHU14	EC0204.22	AA	BB	BB
CHU14	EC0204.23	AA	AA	AA
CHU14	EC0204.24	AA	AB	AB
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CHU14	EC0204.27	AB	AB	AA
CHU14	EC0204.28	AA	AB	BB
CHU14	EC0204.29	AA	AB	AB
CHU14	EC0204.30	AA	AB	AB

CHU15	EC0203.01	AA	AA	AB
CHU15	EC0203.02	AA	AA	AA
CHU15	EC0203.03	AA	AA	AA
CHU15	EC0203.04	AA	AA	AA
CHU15	EC0203.05	AA	AA	AA
CHU15	EC0203.06	AA	AA	AA
CHU15	EC0203.07	AB	AB	AB
CHU15	EC0203.08	AA	AB	AB
CHU15	EC0203.09	AA	AA	AB
CHU15	EC0203.10	AA	AA	AA
CHU15	EC0203.11	AA	AB	AB
CHU15	EC0203.12	AA	AB	AB
CHU15	EC0203.13	AA	AA	AA
CHU15	EC0203.14	AB	AB	AB
CHU15	EC0203.15	AB	AB	AA
CHU15	EC0203.16	AA	AA	AA
CHU15	EC0203.17	AB	AB	AB
CHU15	EC0203.18	AB	AA	BB
CHU15	EC0203.19	AA	AA	AA
CHU15	EC0203.20	AA	AB	AA
CHU15	EC0203.21	AB	AA	AA
CHU15	EC0203.22	AA	AA	AA
CHU15	EC0203.23	AA	AA	AA
CHU15	EC0203.24	AB	AB	BB
CHU15	EC0203.25	AA	AA	AA
CHU15	EC0203.26	AA	AA	AB
CHU15	EC0203.27	AB	AB	BB
CHU15	EC0203.28	AA	AB	AA
CHU15	EC0203.29	AA	BB	BB
CHU15	EC0203.30	AB	AB	AB

CHU16	EC0202.01	AA	AA	AB
CHU16	EC0202.02	AA	AA	AA
CHU16	EC0202.03	AA	AA	AA
CHU16	EC0202.04	AA	AA	AA
CHU16	EC0202.05	AA	AA	AA
CHU16	EC0202.06	AA	AA	AA
CHU16	EC0202.07	AA	AA	AA
CHU16	EC0202.08	AA	AA	AA
CHU16	EC0202.09	AA	AA	AA
CHU16	EC0202.10	AA	AA	AA
CHU16	EC0202.11	AA	AA	AA

CHU16	EC0202.12	AA	AA	AA
CHU16	EC0202.13	AA	AA	AA
CHU16	EC0202.14	AA	AA	AA
CHU16	EC0202.15	AA	AA	AA
CHU16	EC0202.16	AA	AA	AA
CHU16	EC0202.17	AA	AA	AA
CHU16	EC0202.18	AA	AA	BB
CHU16	EC0202.19	AA	AA	AA
CHU16	EC0202.20	AA	AA	AB
CHU16	EC0202.21	AA	AA	AA
CHU16	EC0202.22	AA	AA	AA
CHU16	EC0202.23	AA	AA	AA
CHU16	EC0202.24	AA	AA	AA
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CHU16	EC0202.26	AA	AA	AA
CHU16	EC0202.27	AA	AA	AA
CHU16	EC0202.28	AA	AA	AA
CHU16	EC0202.29	AA	AA	BB
CHU16	EC0202.30	AA	AA	AA

CHU17	EC0201.01	AA	AA	AA
CHU17	EC0201.02	AA	AA	AA
CHU17	EC0201.03	AA	AA	AA
CHU17	EC0201.04	AA	AA	AA
CHU17	EC0201.05	AA	AA	AA
CHU17	EC0201.06	AA	AA	AA
CHU17	EC0201.07	AA	AA	AA
CHU17	EC0201.08	AA	AA	AA
CHU17	EC0201.09	AA	AA	AA
CHU17	EC0201.10	AA	AA	AA
CHU17	EC0201.11	AA	AA	AA
CHU17	EC0201.12	AA	AA	AA
CHU17	EC0201.13	AA	AA	AA
CHU17	EC0201.14	AA	AA	AA
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CHU17	EC0201.16	AA	AA	AA
CHU17	EC0201.17	AA	AA	AA
CHU17	EC0201.18	AA	AA	AA
CHU17	EC0201.19	AA	AA	AA
CHU17	EC0201.20	AA	AA	AA
CHU17	EC0201.21	AA	AA	AA
CHU17	EC0201.22	AA	AA	AA
CHU17	EC0201.23	AA	AA	AA
CHU17	EC0201.24	AA	AA	AA

CHU17	EC0201.25	AA	AA	AA
CHU17	EC0201.26	AA	AA	AB
CHU17	EC0201.27	AA	AA	AA
CHU17	EC0201.28	AA	AA	AA
CHU17	EC0201.29	AA	AA	AA
CHU17	EC0201.30	AA	AA	AA

APPENDIX VI.

CYPRINODON COLLECTION FOR AUGUST 2002

Appendix VI. *Cyprinodon* collection for August 2002

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0231.01	BB	BB	BB
CHU01	EC0231.02	BB	BB	BB
CHU01	EC0231.03	BB	BB	BB
CHU01	EC0231.04	BB	BB	BB
CHU01	EC0231.05	BB	BB	BB
CHU01	EC0231.06	BB	BB	BB
CHU01	EC0231.07	BB	BB	BB
CHU01	EC0231.08	BB	BB	BB
CHU01	EC0231.09	BB	BB	BB
CHU01	EC0231.10	BB	BB	BB
CHU01	EC0231.11	BB	BB	??
CHU01	EC0231.12	BB	BB	BB
CHU01	EC0231.13	BB	BB	BB
CHU01	EC0231.14	BB	BB	BB
CHU01	EC0231.15	BB	BB	BB
CHU01	EC0231.16	BB	BB	BB
CHU01	EC0231.17	BB	BB	BB
CHU01	EC0231.18	BB	BB	BB
CHU01	EC0231.19	BB	BB	BB
CHU01	EC0231.20	BB	BB	BB
CHU01	EC0231.21	BB	BB	BB
CHU01	EC0231.22	BB	BB	BB
CHU01	EC0231.23	BB	BB	??
CHU01	EC0231.24	BB	BB	BB
CHU01	EC0231.25	BB	BB	BB
CHU01	EC0231.26	BB	BB	BB
CHU01	EC0231.27	BB	BB	BB
CHU01	EC0231.28	BB	BB	BB
CHU01	EC0231.29	BB	BB	BB
CHU01	EC0231.30	BB	BB	BB
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CHU02	EC0232.03	BB	BB	BB
CHU02	EC0232.04	BB	BB	BB
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CHU02	EC0232.07	BB	BB	BB
CHU02	EC0232.08	BB	BB	BB
CHU02	EC0232.09	BB	BB	BB

CHU02	EC0232.10	BB	BB	BB
CHU02	EC0232.11	BB	BB	BB
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CHU02	EC0232.13	BB	BB	BB
CHU02	EC0232.14	BB	BB	BB
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CHU02	EC0232.24	BB	BB	BB
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CHU02	EC0232.26	BB	BB	BB
CHU02	EC0232.27	??	BB	BB
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CHU02	EC0232.29	BB	BB	BB
CHU02	EC0232.30	BB	BB	BB

CHU03	EC0233.01	BB	BB	BB
CHU03	EC0233.02	BB	BB	BB
CHU03	EC0233.03	BB	BB	BB
CHU03	EC0233.04	BB	BB	BB
CHU03	EC0233.05	BB	BB	BB
CHU03	EC0233.06	BB	BB	BB
CHU03	EC0233.07	BB	BB	BB
CHU03	EC0233.08	BB	BB	BB
CHU03	EC0233.09	BB	??	BB
CHU03	EC0233.10	BB	BB	BB
CHU03	EC0233.11	BB	BB	BB
CHU03	EC0233.12	BB	BB	BB
CHU03	EC0233.13	BB	BB	BB
CHU03	EC0233.14	BB	BB	BB
CHU03	EC0233.15	BB	BB	BB
CHU03	EC0233.16	BB	BB	BB
CHU03	EC0233.17	BB	BB	BB
CHU03	EC0233.18	BB	BB	BB
CHU03	EC0233.19	BB	BB	BB
CHU03	EC0233.20	BB	BB	BB
CHU03	EC0233.21	BB	BB	BB
CHU03	EC0233.22	BB	BB	BB

CHU03	EC0233.23	BB	BB	BB
CHU03	EC0233.24	BB	BB	BB
CHU03	EC0233.25	BB	BB	BB
CHU03	EC0233.26	BB	BB	BB
CHU03	EC0233.27	BB	BB	BB
CHU03	EC0233.28	BB	BB	BB
CHU03	EC0233.29	BB	BB	BB
CHU03	EC0233.30	BB	BB	BB
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CHU04	EC0234.02	BB	BB	BB
CHU04	EC0234.03	BB	BB	BB
CHU04	EC0234.04	AB	BB	BB
CHU04	EC0234.05	BB	BB	??
CHU04	EC0234.06	BB	BB	BB
CHU04	EC0234.07	AA	AA	??
CHU04	EC0234.08	AA	BB	AA
CHU04	EC0234.09	BB	BB	BB
CHU04	EC0234.10	AB	??	BB
CHU04	EC0234.11	AB	??	BB
CHU04	EC0234.12	BB	??	BB
CHU04	EC0234.13	BB	??	BB
CHU04	EC0234.14	BB	BB	BB
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CHU04	EC0234.17	BB	BB	BB
CHU04	EC0234.18	BB	BB	BB
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CHU04	EC0234.20	BB	BB	??
CHU04	EC0234.21	BB	BB	BB
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CHU04	EC0234.26	BB	BB	BB
CHU04	EC0234.27	BB	BB	??
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CHU05	EC0235.05	AB	AB	BB
CHU05	EC0235.06	AB	BB	??
CHU05	EC0235.07	AB	BB	BB
CHU05	EC0235.08	AB	AB	??
CHU05	EC0235.09	AB	AB	??
CHU05	EC0235.10	AB	BB	BB
CHU05	EC0235.11	BB	BB	??
CHU05	EC0235.12	BB	AB	BB
CHU05	EC0235.13	BB	BB	AB
CHU05	EC0235.14	BB	BB	BB
CHU05	EC0235.15	AB	AB	BB
CHU05	EC0235.16	BB	BB	BB
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CHU05	EC0235.18	BB	BB	BB
CHU05	EC0235.19	BB	AB	BB
CHU05	EC0235.20	BB	BB	??
CHU05	EC0235.21	AA	AA	AA
CHU05	EC0235.22	BB	BB	AB
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CHU05	EC0235.24	BB	BB	BB
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CHU05	EC0235.27	BB	BB	BB
CHU05	EC0235.28	BB	BB	??
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CHU05	EC0235.30	AA	AA	AA
CHU06	EC0236.01	AA	AA	AA
CHU06	EC0236.02	AA	AA	AA
CHU06	EC0236.03	AA	AA	AA
CHU06	EC0236.04	AA	AA	AA
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CHU06	EC0236.07	AA	AA	AA
CHU06	EC0236.08	AA	AA	AA
CHU06	EC0236.09	AA	AA	AA
CHU06	EC0236.10	AA	AA	AA
CHU06	EC0236.11	AA	AA	AA
CHU06	EC0236.12	AA	AA	AA
CHU06	EC0236.13	AA	AA	AA
CHU06	EC0236.14	AA	AA	AA
CHU06	EC0236.15	AB	AA	AA
CHU06	EC0236.16	AA	AA	AA

CHU06	EC0236.17	AA	AA	AA
CHU06	EC0236.18	AA	AA	AA
CHU06	EC0236.19	AA	AA	AA
CHU06	EC0236.20	AA	AA	AA
CHU06	EC0236.21	AA	AA	AA
CHU06	EC0236.22	AA	AA	AA
CHU06	EC0236.23	AA	AA	AA
CHU06	EC0236.24	AA	AA	AA
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CHU07	EC0237.13	AA	AA	AA
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CHU07	EC0237.16	AA	AA	AA
CHU07	EC0237.17	AA	AA	AA
CHU07	EC0237.18	AA	AA	AA
CHU07	EC0237.19	AA	AA	AA
CHU07	EC0237.20	AA	AA	AA
CHU07	EC0237.21	AA	AB	AB
CHU07	EC0237.22	AA	AA	AA
CHU07	EC0237.23	AA	AA	AA
CHU07	EC0237.24	AA	AA	AA
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CHU07	EC0237.27	AA	AA	AA
CHU07	EC0237.28	AA	AA	AA
CHU07	EC0237.29	BB	BB	BB

CHU07	EC0237.30	AA	AA	AB
CHU08	EC0238.01	AB	AB	BB
CHU08	EC0238.02	AB	AB	BB
CHU08	EC0238.03	BB	??	AB
CHU08	EC0238.04	AA	AA	AA
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CHU08	EC0238.06	AA	AA	BB
CHU08	EC0238.07	AA	AA	AA
CHU08	EC0238.08	BB	BB	BB
CHU08	EC0238.09	AA	AA	AA
CHU08	EC0238.10	BB	AB	BB
CHU08	EC0238.11	AA	AB	AA
CHU08	EC0238.12	AA	AA	AA
CHU08	EC0238.13	AA	AA	AA
CHU08	EC0238.14	AA	AA	AA
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CHU08	EC0238.16	AA	AA	AA
CHU08	EC0238.17	AB	AB	AB
CHU08	EC0238.18	AA	AA	AA
CHU08	EC0238.19	AB	AB	AA
CHU08	EC0238.20	AA	AA	AA
CHU08	EC0238.21	AA	AA	AA
CHU08	EC0238.22	AA	AA	AA
CHU08	EC0238.23	AA	AA	AA
CHU08	EC0238.24	AA	AA	AA
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CHU08	EC0238.27	AA	AA	AA
CHU08	EC0238.28	BB	BB	BB
CHU08	EC0238.29	AA	??	AA
CHU08	EC0238.30	BB	BB	BB
CHU09	EC0239.01	AA	AA	AA
CHU09	EC0239.02	BB	BB	BB
CHU09	EC0239.03	AB	AB	BB
CHU09	EC0239.04	AA	BB	AB
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CHU09	EC0239.07	AA	AB	BB
CHU09	EC0239.08	AB	BB	BB
CHU09	EC0239.09	AA	AA	AA
CHU09	EC0239.10	AA	AA	AA

CHU09	EC0239.11	AA	AA	AA
CHU09	EC0239.12	AA	??	AA
CHU09	EC0239.13	BB	AB	AB
CHU09	EC0239.14	AA	??	AA
CHU09	EC0239.15	AA	AA	AA
CHU09	EC0239.16	AA	AA	AB
CHU09	EC0239.17	AA	AA	AA
CHU09	EC0239.18	AA	AA	AA
CHU09	EC0239.19	AA	AA	AA
CHU09	EC0239.20	AA	AA	AA
CHU09	EC0239.21	AA	AA	AA
CHU09	EC0239.22	AA	AA	AA
CHU09	EC0239.23	AA	AA	AB
CHU09	EC0239.24	AA	AA	AA
CHU09	EC0239.25	AA	AA	AA
CHU09	EC0239.26	AA	AA	AA
CHU09	EC0239.27	BB	BB	AB
CHU09	EC0239.28	AA	AA	AB
CHU09	EC0239.29	AA	AA	AA
CHU09	EC0239.30	AB	AB	AB

CHU10	EC0240.01	AA	AA	AA
CHU10	EC0240.02	AB	BB	BB
CHU10	EC0240.03	AA	AA	AA
CHU10	EC0240.04	AA	AA	AA
CHU10	EC0240.05	BB	BB	AA
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CHU10	EC0240.07	AA	AA	AA
CHU10	EC0240.08	AA	AA	AA
CHU10	EC0240.09	AA	AA	AA
CHU10	EC0240.10	AA	AA	AA
CHU10	EC0240.11	AA	AA	AA
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CHU10	EC0240.16	AA	AA	AA
CHU10	EC0240.17	AA	AA	AA
CHU10	EC0240.18	AA	AA	AA
CHU10	EC0240.19	AA	AA	AA
CHU10	EC0240.20	AA	AA	AA
CHU10	EC0240.21	AA	AA	AA
CHU10	EC0240.22	AA	AA	AA
CHU10	EC0240.23	AA	AA	AA

CHU10	EC0240.24	AB	AB	AA
CHU10	EC0240.25	AA	AA	AA
CHU10	EC0240.26	AA	AA	AB
CHU10	EC0240.27	AA	AA	AB
CHU10	EC0240.28	AA	AA	AA
CHU10	EC0240.29	AA	AA	AA
CHU10	EC0240.30	AA	AA	AA
CHU11	EC0241.01	BB	BB	BB
CHU11	EC0241.02	??	AA	AB
CHU11	EC0241.03	AA	BB	AB
CHU11	EC0241.04	AA	AA	AA
CHU11	EC0241.05	??	??	AA
CHU11	EC0241.06	AA	AA	AA
CHU11	EC0241.07	AA	AA	AA
CHU11	EC0241.08	AA	AA	AB
CHU11	EC0241.09	AB	??	AB
CHU11	EC0241.10	AA	AA	AA
CHU11	EC0241.11	BB?	AB	AB
CHU11	EC0241.12	AA	AB	AA
CHU11	EC0241.13	AA	AA	AB
CHU11	EC0241.14	AA	AA	AA
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CHU11	EC0241.17	AA	AA	AA
CHU11	EC0241.18	AB	AB	BB
CHU11	EC0241.19	BB	AB	BB
CHU11	EC0241.20	??	AA	AA
CHU11	EC0241.21	AA	BB	AA
CHU11	EC0241.22	AA	AA	AA
CHU11	EC0241.23	AB	AB	AB
CHU11	EC0241.24	??	AA	AA
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CHU12	EC0242.01	AA	AA	AA
CHU12	EC0242.02	AA	AA	AA
CHU12	EC0242.03	AA	AB	AA
CHU12	EC0242.04	AA	AA	AA

CHU12	EC0242.05	AA	AA	AA
CHU12	EC0242.06	??	AB	AA
CHU12	EC0242.07	AA	AA	AA
CHU12	EC0242.08	AA	AA	AA
CHU12	EC0242.09	AA	AB	AA
CHU12	EC0242.10	AA	AA	AB
CHU12	EC0242.11	AA	AA	AA
CHU12	EC0242.12	AA	AA	AA
CHU12	EC0242.13	AA	AA	AA
CHU12	EC0242.14	AA	AB	AA
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CHU12	EC0242.17	AA	AA	AA
CHU12	EC0242.18	AA	AB	AA
CHU12	EC0242.19	AA	AA	AA
CHU12	EC0242.20	AA	AA	AA
CHU12	EC0242.21	AA	AB	AA
CHU12	EC0242.22	AA	AA	AA
CHU12	EC0242.23	AA	AA	AA
CHU12	EC0242.24	AB	AB	AB
CHU12	EC0242.25	AA	AA	AA
CHU12	EC0242.26	AA	AA	AB
CHU12	EC0242.27	AA	AA	AA
CHU12	EC0242.28	AA	AB	AA
CHU12	EC0242.29	AA	AB	AA
CHU12	EC0242.30	AA	AA	AA

CHU13	EC0243.01	AB	AB	BB
CHU13	EC0243.02	AA	BB	BB
CHU13	EC0243.03	AB	BB	AB
CHU13	EC0243.04	AB	AB	AB
CHU13	EC0243.05	BB	AB	AB
CHU13	EC0243.06	AA	AB	AB
CHU13	EC0243.07	AB	AB	BB
CHU13	EC0243.08	AA	AB	BB
CHU13	EC0243.09	AB	BB	AB
CHU13	EC0243.10	BB	BB	BB
CHU13	EC0243.11	AB	AB	AA
CHU13	EC0243.12	AA	BB	AB
CHU13	EC0243.13	BB	AB	AA
CHU13	EC0243.14	AA	AB	AA
CHU13	EC0243.15	AB	AB	AB
CHU13	EC0243.16	BB	AB	BB
CHU13	EC0243.17	BB	BB	AB

CHU13	EC0243.18	AA	AA	AA
CHU13	EC0243.19	AA	AA	AA
CHU13	EC0243.20	BB	??	AB
CHU13	EC0243.21	AA	AB	AB
CHU13	EC0243.22	BB	AB	AB
CHU13	EC0243.23	AA	AA	AB
CHU13	EC0243.24	AB	BB	AA
CHU13	EC0243.25	AB	BB	AB
CHU13	EC0243.26	AA	AB	AB
CHU13	EC0243.27	AA	AB	AB
CHU13	EC0243.28	AA	BB	BB
CHU13	EC0243.29	AB	AB	BB
CHU13	EC0243.30	AA	BB	AB
CHU14	EC0244.01	AA	AA	BB
CHU14	EC0244.02	AB	AA	AB
CHU14	EC0244.03	AA	AA	AA
CHU14	EC0244.04	AA	AA	AA
CHU14	EC0244.05	AA	AA	AA
CHU14	EC0244.06	AA	??	AB
CHU14	EC0244.07	AA	AA	AA
CHU14	EC0244.08	AA	AA	AA
CHU14	EC0244.09	AA	AA	AA
CHU14	EC0244.10	AA	AA	AA
CHU14	EC0244.11	AA	AA	AA
CHU14	EC0244.12	AA	AA	AA
CHU14	EC0244.13	AA	AA	AA
CHU14	EC0244.14	AA	AA	AA
CHU14	EC0244.15	AB	AA	AB
CHU14	EC0244.16	AA	AA	AA
CHU14	EC0244.17	AA	AA	AA
CHU14	EC0244.18	AA	AA	AA
CHU14	EC0244.19	AA	AA	AA
CHU14	EC0244.20	AA	AA	AB
CHU14	EC0244.21	AA	AA	AA
CHU14	EC0244.22	AA	AA	AA
CHU14	EC0244.23	AA	AB	AB
CHU14	EC0244.24	BB	AA	AB
CHU14	EC0244.25	AA	AA	AA
CHU14	EC0244.26	AA	AA	AA
CHU14	EC0244.27	AA	AA	AA
CHU14	EC0244.28	AA	AA	AA
CHU14	EC0244.29	AA	AA	AA
CHU14	EC0244.30	AA	AA	AA

CHU15	EC0245.01	AA	??	AA
CHU15	EC0245.02	AA	AB?	AB
CHU15	EC0245.03	AA	AA	AB
CHU15	EC0245.04	AA	AA	??
CHU15	EC0245.05	AA	AA	AA
CHU15	EC0245.06	AA	AA	AA
CHU15	EC0245.07	AA	??	AA
CHU15	EC0245.08	AA	AA	AA
CHU15	EC0245.09	AA	AA	AA
CHU15	EC0245.10	AA	??	AA
CHU15	EC0245.11	AA	AA	AB
CHU15	EC0245.12	AA	??	AA
CHU15	EC0245.13	AA	??	AA
CHU15	EC0245.14	AA	AA	AB
CHU15	EC0245.15	AA	??	AA
CHU15	EC0245.16	AA	AA	AA
CHU15	EC0245.17	AA	AA	AA
CHU15	EC0245.18	AA	AA	AA
CHU15	EC0245.19	AA	??	AA
CHU15	EC0245.20	AA	AA	AA
CHU15	EC0245.21	AA	AA	AA
CHU15	EC0245.22	AA	AA	AA
CHU15	EC0245.23	AA	AA	AA
CHU15	EC0245.24	AA	AA	AB
CHU15	EC0245.25	AA	AA	AA
CHU15	EC0245.26	AA	??	AA
CHU15	EC0245.27	AA	AA	AA
CHU15	EC0245.28	AA	AA	AA
CHU15	EC0245.29	AA	AA	AA
CHU15	EC0245.30	AA	??	AA

CHU16	EC0246.01	AA	AA	AB
CHU16	EC0246.02	AA	AA	AA
CHU16	EC0246.03	AA	AA	AA
CHU16	EC0246.04	AA	AA	AA
CHU16	EC0246.05	AA	AA	AA
CHU16	EC0246.06	AA	AA	AA
CHU16	EC0246.07	AA	AA	AB
CHU16	EC0246.08	AA	AA	AA
CHU16	EC0246.09	AA	AA	AA
CHU16	EC0246.10	AA	AA	AA
CHU16	EC0246.11	AA	AA	AA

CHU16	EC0246.12	AA	AA	AA
CHU16	EC0246.13	AA	AA	AB
CHU16	EC0246.14	AA	AA	AA
CHU16	EC0246.15	AA	AA	AA
CHU16	EC0246.16	AA	AA	AA
CHU16	EC0246.17	AA	AA	AA
CHU16	EC0246.18	AA	AA	AA
CHU16	EC0246.19	AA	AA	AA
CHU16	EC0246.20	AA	AA	AA
CHU16	EC0246.21	AA	AA	AA
CHU16	EC0246.22	AA	AA	AA
CHU16	EC0246.23	AA	AA	AA
CHU16	EC0246.24	AA	AA	AB
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CHU16	EC0246.27	AA	AA	AA
CHU16	EC0246.28	AA	AA	AA
CHU16	EC0246.29	AA	AA	AA
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CHU17	EC0247.02	AA	AA	AA
CHU17	EC0247.03	AA	AA	AB
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CHU17	EC0247.05	AA	AA	AA
CHU17	EC0247.06	AA	AA	AA
CHU17	EC0247.07	AA	AA	AA
CHU17	EC0247.08	AA	AA	AA
CHU17	EC0247.09	AA	AA	AA
CHU17	EC0247.10	AA	AA	AA
CHU17	EC0247.11	AA	AA	AA
CHU17	EC0247.12	AA	AA	AA
CHU17	EC0247.13	AA	AA	AB
CHU17	EC0247.14	AB	AA	AB
CHU17	EC0247.15	AA	AA	AB
CHU17	EC0247.16	AA	AA	AA
CHU17	EC0247.17	AA	AA	AA
CHU17	EC0247.18	AA	AA	AA
CHU17	EC0247.19	AA	AA	AA
CHU17	EC0247.20	AA	AA	AA
CHU17	EC0247.21	AB	AA	AA
CHU17	EC0247.22	AA	AA	AB
CHU17	EC0247.23	AA	AA	AA
CHU17	EC0247.24	AA	AA	AA

CHU17	EC0247.25	AA	AA	AA
CHU17	EC0247.26	AA	AA	AA
CHU17	EC0247.27	AA	AA	AA
CHU17	EC0247.28	AA	AA	AA
CHU17	EC0247.29	AA	AA	AB
CHU17	EC0247.30	AA	AA	AA

APPENDIX VII.

CYPRINODON COLLECTION FOR JANUARY 2003

Appendix VII. *Cyprinodon* collection for January 2003

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0301.01	BB	BB	BB
CHU01	EC0301.02	BB	BB	BB
CHU01	EC0301.03	BB	BB	BB
CHU01	EC0301.04	BB	BB	BB
CHU01	EC0301.05	BB	BB	BB
CHU01	EC0301.06	BB	BB	BB
CHU01	EC0301.07	BB	BB	BB
CHU01	EC0301.08	BB	BB	BB
CHU01	EC0301.09	BB	BB	BB
CHU01	EC0301.10	??	BB	BB
CHU01	EC0301.11	BB	BB	BB
CHU01	EC0301.12	BB	BB	BB
CHU01	EC0301.13	BB	BB	BB
CHU01	EC0301.14	BB	BB	BB
CHU01	EC0301.15	BB	BB	BB
CHU01	EC0301.16	BB	BB	BB
CHU01	EC0301.17	BB	BB	BB
CHU01	EC0301.18	BB	BB	BB
CHU01	EC0301.19	BB	BB	BB
CHU01	EC0301.20	BB	BB	BB
CHU01	EC0301.21	BB	BB	BB
CHU01	EC0301.22	BB	BB	BB
CHU01	EC0301.23	BB	BB	BB
CHU01	EC0301.24	BB	BB	BB
CHU01	EC0301.25	BB	BB	BB
CHU01	EC0301.26	BB	BB	BB
CHU01	EC0301.27	BB	BB	BB
CHU01	EC0301.28	BB	BB	BB
CHU01	EC0301.29	BB	BB	BB
CHU01	EC0301.30	BB	BB	BB
CHU02	EC0302.01	BB	BB	BB
CHU02	EC0302.02	BB	BB	BB
CHU02	EC0302.03	BB	BB	BB
CHU02	EC0302.04	BB	BB	BB
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CHU02	EC0302.06	BB	BB	BB
CHU02	EC0302.07	BB	BB	BB
CHU02	EC0302.08	BB	BB	BB
CHU02	EC0302.09	BB	BB	BB

CHU02	EC0302.10	BB	BB	BB
CHU02	EC0302.11	BB	BB	BB
CHU02	EC0302.12	BB	BB	BB
CHU02	EC0302.13	BB	BB	BB
CHU02	EC0302.14	BB	BB	BB
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CHU02	EC0302.16	BB	BB	BB
CHU02	EC0302.17	BB	BB	BB
CHU02	EC0302.18	BB	BB	BB
CHU02	EC0302.19	BB	BB	BB
CHU02	EC0302.20	BB	BB	BB
CHU02	EC0302.21	BB	BB	BB
CHU02	EC0302.22	BB	BB	BB
CHU02	EC0302.23	??	BB	BB
CHU02	EC0302.24	BB	BB	BB
CHU02	EC0302.25	BB	BB	BB
CHU02	EC0302.26	BB	BB	BB
CHU02	EC0302.27	BB	BB	BB
CHU02	EC0302.28	BB	BB	BB
CHU02	EC0302.29	BB	BB	BB
CHU02	EC0302.30	BB	BB	BB

CHU03	EC0303.01	BB	BB	BB
CHU03	EC0303.02	BB	BB	BB
CHU03	EC0303.03	BB	BB	BB
CHU03	EC0303.04	BB	BB	BB
CHU03	EC0303.05	BB	BB	BB
CHU03	EC0303.06	BB	BB	BB
CHU03	EC0303.07	BB	BB	BB
CHU03	EC0303.08	BB	BB	BB
CHU03	EC0303.09	BB	BB	BB
CHU03	EC0303.10	BB	BB	BB
CHU03	EC0303.11	BB	BB	BB
CHU03	EC0303.12	BB	BB	BB
CHU03	EC0303.13	BB	BB	BB
CHU03	EC0303.14	BB	BB	BB
CHU03	EC0303.15	BB	BB	BB
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CHU03	EC0303.17	BB	BB	BB
CHU03	EC0303.18	BB	BB	BB
CHU03	EC0303.19	BB	BB	BB
CHU03	EC0303.20	BB	BB	BB
CHU03	EC0303.21	BB	BB	BB
CHU03	EC0303.22	BB	BB	BB

CHU03	EC0303.23	BB	BB	BB
CHU03	EC0303.24	BB	BB	BB
CHU03	EC0303.25	BB	BB	BB
CHU03	EC0303.26	BB	BB	BB
CHU03	EC0303.27	BB	BB	BB
CHU03	EC0303.28	BB	BB	BB
CHU03	EC0303.29	BB	BB	BB
CHU03	EC0303.30	BB	BB	BB
CHU04	EC0304.01	BB	BB	AB
CHU04	EC0304.02	BB	BB	BB
CHU04	EC0304.03	AB	BB	BB
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CHU04	EC0304.06	BB	BB	AB
CHU04	EC0304.07	BB	BB	AB
CHU04	EC0304.08	AB	BB	BB
CHU04	EC0304.09	BB	BB	BB
CHU04	EC0304.10	BB	BB	AB
CHU04	EC0304.11	BB	BB	AB
CHU04	EC0304.12	BB	BB	BB
CHU04	EC0304.13	BB	BB	BB
CHU04	EC0304.14	BB	BB	BB
CHU04	EC0304.15	BB	BB	BB
CHU04	EC0304.16	BB	BB	BB
CHU04	EC0304.17	BB	BB	BB
CHU04	EC0304.18	BB	BB	BB
CHU04	EC0304.19	BB	BB	BB
CHU04	EC0304.20	BB	BB	BB
CHU04	EC0304.21	BB	BB	BB
CHU04	EC0304.22	BB	BB	BB
CHU04	EC0304.23	BB	BB	BB
CHU04	EC0304.24	BB	BB	BB
CHU04	EC0304.25	AA	BB	BB
CHU04	EC0304.26	BB	BB	BB
CHU04	EC0304.27	BB	BB	BB
CHU04	EC0304.28	BB	BB	BB
CHU04	EC0304.29	BB	BB	BB
CHU04	EC0304.30	BB	BB	BB
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CHU05	EC0305.02	BB	BB	BB
CHU05	EC0305.03	BB	BB	BB

CHU05	EC0305.04	BB	BB	BB
CHU05	EC0305.05	BB	BB	BB
CHU05	EC0305.06	AB	BB	BB
CHU05	EC0305.07	AB	BB	AB
CHU05	EC0305.08	BB	BB	BB
CHU05	EC0305.09	BB	BB	BB
CHU05	EC0305.10	AB	BB	BB
CHU05	EC0305.11	BB	BB	BB
CHU05	EC0305.12	BB	BB	BB
CHU05	EC0305.13	AB	BB	AB
CHU05	EC0305.14	BB	BB	BB
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CHU05	EC0305.17	BB	BB	BB
CHU05	EC0305.18	BB	BB	BB
CHU05	EC0305.19	BB	BB	BB
CHU05	EC0305.20	BB	BB	BB
CHU05	EC0305.21	BB	BB	BB
CHU05	EC0305.22	BB	BB	BB
CHU05	EC0305.23	BB	BB	BB
CHU05	EC0305.24	AA	BB	AB
CHU05	EC0305.25	BB	BB	BB
CHU05	EC0305.26	AB	BB	AB
CHU05	EC0305.27	AB	BB	AB
CHU05	EC0305.28	BB	BB	BB
CHU05	EC0305.29	BB	BB	BB
CHU05	EC0305.30	AB	BB	AA
CHU06	EC0306.01	AA	AA	AA
CHU06	EC0306.02	AA	AA	AA
CHU06	EC0306.03	AA	AA	AA
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CHU06	EC0306.05	AA	AA	AA
CHU06	EC0306.06	AA	AA	AA
CHU06	EC0306.07	AA	AA	AA
CHU06	EC0306.08	AA	AA	AA
CHU06	EC0306.09	AA	AB	AA
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CHU06	EC0306.11	AA	AA	AA
CHU06	EC0306.12	AA	AB	AA
CHU06	EC0306.13	AA	AA	AA
CHU06	EC0306.14	AA	AA	AA
CHU06	EC0306.15	AA	AA	AA
CHU06	EC0306.16	AA	AA	AA

CHU06	EC0306.17	AA	AA	AA
CHU06	EC0306.18	AA	AA	AA
CHU06	EC0306.19	AA	AA	AA
CHU06	EC0306.20	AA	AA	AA
CHU06	EC0306.21	AA	AA	AA
CHU06	EC0306.22	AA	AA	AA
CHU06	EC0306.23	AA	AB	AA
CHU06	EC0306.24	AA	AA	AA
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CHU07	EC0307.03	AA	AA	AA
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CHU07	EC0307.05	AA	AA	AA
CHU07	EC0307.06	BB	BB	BB
CHU07	EC0307.07	AA	AA	AA
CHU07	EC0307.08	AA	AA	AA
CHU07	EC0307.09	AA	AA	AA
CHU07	EC0307.10	AA	AA	AA
CHU07	EC0307.11	AA	AA	AA
CHU07	EC0307.12	AA	AA	AA
CHU07	EC0307.13	AB	AB	AB
CHU07	EC0307.14	AA	AA	AA
CHU07	EC0307.15	AA	AA	AA
CHU07	EC0307.16	AA	AA	AA
CHU07	EC0307.17	AA	AA	AA
CHU07	EC0307.18	AA	AA	AA
CHU07	EC0307.19	AA	AA	??
CHU07	EC0307.20	AA	AA	??
CHU07	EC0307.21	AB	AA	AA
CHU07	EC0307.22	BB	BB	??
CHU07	EC0307.23	AB	AB	AB
CHU07	EC0307.24	AA	AA	AA
CHU07	EC0307.25	AA	AA	AA
CHU07	EC0307.26	AA	AA	??
CHU07	EC0307.27	AA	AA	AA
CHU07	EC0307.28	AA	AA	AA
CHU07	EC0307.29	AA	AA	AA

CHU07	EC0307.30	AA	??	AA
CHU08	EC0308.01	BB	BB	BB
CHU08	EC0308.02	BB	BB	BB
CHU08	EC0308.03	AB	AA	AB
CHU08	EC0308.04	AA	BB	AB
CHU08	EC0308.05	BB	AB	AB
CHU08	EC0308.06	AB	BB	BB
CHU08	EC0308.07	BB	BB	BB
CHU08	EC0308.08	BB	AB	BB
CHU08	EC0308.09	AA	AA	AA
CHU08	EC0308.10	BB	BB	BB
CHU08	EC0308.11	AA	AA	AA
CHU08	EC0308.12	AA	AA	AA
CHU08	EC0308.13	AB	BB	AB
CHU08	EC0308.14	AA	AA	AA
CHU08	EC0308.15	AB	BB	BB
CHU08	EC0308.16	AB	AB	BB
CHU08	EC0308.17	BB	AB	AB
CHU08	EC0308.18	AB	BB	AB
CHU08	EC0308.19	AB	AB	AB
CHU08	EC0308.20	AA	AA	AA
CHU08	EC0308.21	AB	AB	AB
CHU08	EC0308.22	AB	BB	BB
CHU08	EC0308.23	BB	AB	BB
CHU08	EC0308.24	AA	AB	AB
CHU08	EC0308.25	BB	BB	BB
CHU08	EC0308.26	AB	AB	AA
CHU08	EC0308.27	AA	AB	AB
CHU08	EC0308.28	AB	AB	AB
CHU08	EC0308.29	NA	NA	NA
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CHU09	EC0309.04	AA	AA	AA
CHU09	EC0309.05	AA	AA	AA
CHU09	EC0309.06	AA	AA	AA
CHU09	EC0309.07	AA	AA	AB
CHU09	EC0309.08	AB	AB	AB
CHU09	EC0309.09	AB	AB	BB
CHU09	EC0309.10	AB	AA	BB

CHU09	EC0309.11	AA	AB	AA
CHU09	EC0309.12	BB	BB	BB
CHU09	EC0309.13	AA	AA	AA
CHU09	EC0309.14	AA	AA	AA
CHU09	EC0309.15	AA	AB	AB
CHU09	EC0309.16	AB	BB	AB
CHU09	EC0309.17	AB	BB	AB
CHU09	EC0309.18	AA	AA	AA
CHU09	EC0309.19	AA	AA	AA
CHU09	EC0309.20	AB	AA	??
CHU09	EC0309.21	AA	AA	AA
CHU09	EC0309.22	AA	AA	AA
CHU09	EC0309.23	AB	AB	BB
CHU09	EC0309.24	AA	BB	BB
CHU09	EC0309.25	AB	AA	AB
CHU09	EC0309.26	AA	AA	AA
CHU09	EC0309.27	AA	AA	AA
CHU09	EC0309.28	BB	AB	AB
CHU09	EC0309.29	BB	BB	AB
CHU09	EC0309.30	AB	AB	AB
CHU10	EC0310.01	AA	AA	AA
CHU10	EC0310.02	AA	AA	AB
CHU10	EC0310.03	BB	BB	AB
CHU10	EC0310.04	AA	AA	AA
CHU10	EC0310.05	AA	AA	AA
CHU10	EC0310.06	BB	BB	AB
CHU10	EC0310.07	AB	AB	AB
CHU10	EC0310.08	AA	AA	AA
CHU10	EC0310.09	AA	AB	AB
CHU10	EC0310.10	AA	AB	AA
CHU10	EC0310.11	BB	BB	BB
CHU10	EC0310.12	AB	BB	BB
CHU10	EC0310.13	AA	AA	AA
CHU10	EC0310.14	AB	BB	BB
CHU10	EC0310.15	AA	AB	AA
CHU10	EC0310.16	AA	AB	AB
CHU10	EC0310.17	BB	BB	BB
CHU10	EC0310.18	AA	AB	AA
CHU10	EC0310.19	AA	AA	AA
CHU10	EC0310.20	AA	AA	AA
CHU10	EC0310.21	BB	BB	AB
CHU10	EC0310.22	AA	AA	AB
CHU10	EC0310.23	BB	BB	BB

CHU10	EC0310.24	AA	BB	BB
CHU10	EC0310.25	AA	AA	AA
CHU10	EC0310.26	AB	AB	AB
CHU10	EC0310.27	AB	AA	AA
CHU10	EC0310.28	AB	AB	AB
CHU10	EC0310.29	AB	AB	AB
CHU10	EC0310.30	BB	BB	BB
CHU11	EC0311.01	AB	AB	AB
CHU11	EC0311.02	AA	AA	AA
CHU11	EC0311.03	AA	AA	AA
CHU11	EC0311.04	AA	AA	AA
CHU11	EC0311.05	AA	AB	AA
CHU11	EC0311.06	AA	AA	AB
CHU11	EC0311.07	AB	AB	AB
CHU11	EC0311.08	AA	AA	AA
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CHU11	EC0311.17	AB	BB	BB
CHU11	EC0311.18	AA	AA	AA
CHU11	EC0311.19	AA	BB	AB
CHU11	EC0311.20	BB	AB	AB
CHU11	EC0311.21	AA	AA	AA
CHU11	EC0311.22	AA	AB	AA
CHU11	EC0311.23	AA	AA	AA
CHU11	EC0311.24	AB	AB	AB
CHU11	EC0311.25	AA	AA	AA
CHU11	EC0311.26	AB	AB	AB
CHU11	EC0311.27	AB	BB	BB
CHU11	EC0311.28	AB	AA	AA
CHU11	EC0311.29	AA	AA	AA
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CHU12	EC0312.01	AA	AA	AA
CHU12	EC0312.02	AA	AA	AA
CHU12	EC0312.03	AA	AA	AA
CHU12	EC0312.04	AA	AA	AA

CHU12	EC0312.05	AA	AA	AA
CHU12	EC0312.06	AA	AA	AA
CHU12	EC0312.07	AA	AA	AA
CHU12	EC0312.08	AA	AA	AA
CHU12	EC0312.09	AA	AA	AA
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CHU12	EC0312.15	AA	AA	AA
CHU12	EC0312.16	AA	AA	AA
CHU12	EC0312.17	AA	AA	AA
CHU12	EC0312.18	AA	AA	AA
CHU12	EC0312.19	AA	AA	AB
CHU12	EC0312.20	AA	??	AA
CHU12	EC0312.21	AA	AA	AA
CHU12	EC0312.22	AA	AA	AA
CHU12	EC0312.23	AB	AA	AB
CHU12	EC0312.24	AA	AB	AA
CHU12	EC0312.25	AB	AA	AB
CHU12	EC0312.26	AA	AA	AA
CHU12	EC0312.27	AA	AB	AA
CHU12	EC0312.28	AA	AA	AA
CHU12	EC0312.29	AA	AA	??
CHU12	EC0312.30	AA	AA	AA

CHU13	EC0313.01	AA	BB	AB
CHU13	EC0313.02	AB	AB	AA
CHU13	EC0313.03	AA	AA	AA
CHU13	EC0313.04	BB	AA	AB
CHU13	EC0313.05	BB	AB	BB
CHU13	EC0313.06	BB	AB	AB
CHU13	EC0313.07	BB	AB	BB
CHU13	EC0313.08	AA	AB	AB
CHU13	EC0313.09	BB	BB	BB
CHU13	EC0313.10	AB	BB	BB
CHU13	EC0313.11	AB	BB	BB
CHU13	EC0313.12	AB	AB	AB
CHU13	EC0313.13	AA	AB	BB
CHU13	EC0313.14	AB	AB	AB
CHU13	EC0313.15	AB	AB	AB
CHU13	EC0313.16	AA	AA	AA
CHU13	EC0313.17	BB	BB	AB

CHU13	EC0313.18	AB	AB	AB
CHU13	EC0313.19	AB	BB	BB
CHU13	EC0313.20	AA	AB	AB
CHU13	EC0313.21	BB	BB	BB
CHU13	EC0313.22	AA	AB	BB
CHU13	EC0313.23	AA	BB	AB
CHU13	EC0313.24	AA	AA	AA
CHU13	EC0313.25	BB	BB	BB
CHU13	EC0313.26	AA	AB	BB
CHU13	EC0313.27	AB	AA	AA
CHU13	EC0313.28	AA	AB	AA
CHU13	EC0313.29	BB	AB	AB
CHU13	EC0313.30	BB	BB	AB
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CHU14	EC0314.02	AA	AA	AA
CHU14	EC0314.03	AA	AA	AA
CHU14	EC0314.04	AA	AA	AB
CHU14	EC0314.05	AA	AA	AA
CHU14	EC0314.06	AA	AA	AA
CHU14	EC0314.07	AA	AA	??
CHU14	EC0314.08	AB	BB	BB
CHU14	EC0314.09	AA	AA	AA
CHU14	EC0314.10	AB	AB	AB
CHU14	EC0314.11	AB	??	BB
CHU14	EC0314.12	AA	AA	AB
CHU14	EC0314.13	AB	BB	BB
CHU14	EC0314.14	AA	AA	BB
CHU14	EC0314.15	AA	AA	AB
CHU14	EC0314.16	AA	AA	AA
CHU14	EC0314.17	AA	AA	AA
CHU14	EC0314.18	AB	BB	BB
CHU14	EC0314.19	AA	AB	AB
CHU14	EC0314.20	AB	AB	AB
CHU14	EC0314.21	AB	BB	BB
CHU14	EC0314.22	AB	BB	BB
CHU14	EC0314.23	BB	BB	BB
CHU14	EC0314.24	BB	BB	AB
CHU14	EC0314.25	AB	AA	AB
CHU14	EC0314.26	AA	AB	AB
CHU14	EC0314.27	AA	AA	AA
CHU14	EC0314.28	BB	AA	AB
CHU14	EC0314.29	AA	AB	BB
CHU14	EC0314.30	BB	??	AB

CHU15	EC0315.01	AA	AA	AA
CHU15	EC0315.02	AA	AA	AA
CHU15	EC0315.03	AA	AA	AA
CHU15	EC0315.04	AA	AA	AA
CHU15	EC0315.05	AA	AA	AA
CHU15	EC0315.06	AA	AB	AA
CHU15	EC0315.07	AA	AA	AA
CHU15	EC0315.08	AA	AA	AA
CHU15	EC0315.09	AA	AA	BB
CHU15	EC0315.10	AA	AA	AA
CHU15	EC0315.11	AA	AA	AA
CHU15	EC0315.12	AA	AA	AA
CHU15	EC0315.13	AA	AA	AA
CHU15	EC0315.14	AA	AA	AA
CHU15	EC0315.15	AA	AA	AA
CHU15	EC0315.16	AA	AA	AA
CHU15	EC0315.17	AA	AA	AA
CHU15	EC0315.18	AB	AB	AB
CHU15	EC0315.19	AA	AA	AB
CHU15	EC0315.20	AB	AA	BB
CHU15	EC0315.21	AA	AA	AA
CHU15	EC0315.22	AA	BB	BB
CHU15	EC0315.23	AA	AA	AA
CHU15	EC0315.24	BB	AB	BB
CHU15	EC0315.25	AA	AA	AA
CHU15	EC0315.26	BB	BB	AB
CHU15	EC0315.27	BB	AB	BB
CHU15	EC0315.28	AB	AB	AA
CHU15	EC0315.29	AA	AA	AB
CHU15	EC0315.30	AA	AB	BB

CHU16	EC0316.01	AA	AA	AB
CHU16	EC0316.02	AA	AA	AA
CHU16	EC0316.03	AA	AA	AA
CHU16	EC0316.04	AA	AA	AA
CHU16	EC0316.05	AA	AA	AA
CHU16	EC0316.06	AA	AA	AA
CHU16	EC0316.07	AA	AA	AA
CHU16	EC0316.08	AA	AA	AA
CHU16	EC0316.09	AA	AA	AA
CHU16	EC0316.10	AA	AA	AA
CHU16	EC0316.11	AA	AA	AA

CHU16	EC0316.12	AA	AA	AA
CHU16	EC0316.13	AA	AA	AA
CHU16	EC0316.14	AA	AA	AB
CHU16	EC0316.15	AA	AA	AA
CHU16	EC0316.16	AA	AA	AA
CHU16	EC0316.17	AA	AA	AA
CHU16	EC0316.18	AA	AA	AA
CHU16	EC0316.19	AA	AA	AA
CHU16	EC0316.20	AA	AA	AA
CHU16	EC0316.21	AA	AA	AA
CHU16	EC0316.22	AA	AA	??
CHU16	EC0316.23	AA	AA	AA
CHU16	EC0316.24	AA	AA	AA
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CHU16	EC0316.27	AA	AA	AA
CHU16	EC0316.28	AA	AA	AA
CHU16	EC0316.29	AA	AA	AA
CHU16	EC0316.30	AA	AA	AA
CHU17	EC0317.01	AA	AA	AA
CHU17	EC0317.02	AA	AA	AA
CHU17	EC0317.03	AA	AB	AA
CHU17	EC0317.04	AA	AA	AA
CHU17	EC0317.05	AA	AA	AA
CHU17	EC0317.06	AA	AA	AB
CHU17	EC0317.07	AA	AA	AA
CHU17	EC0317.08	AA	AA	AA
CHU17	EC0317.09	AA	AA	AA
CHU17	EC0317.10	AA	AA	AA
CHU17	EC0317.11	AA	AA	AA
CHU17	EC0317.12	AA	AA	AA
CHU17	EC0317.13	AA	AA	AA
CHU17	EC0317.14	AA	AA	AA
CHU17	EC0317.15	AA	AA	AA
CHU17	EC0317.16	AA	AA	AA
CHU17	EC0317.17	AA	AA	AA
CHU17	EC0317.18	AA	AA	AA
CHU17	EC0317.19	AA	AA	AA
CHU17	EC0317.20	AA	AA	AB
CHU17	EC0317.21	AA	AA	AA
CHU17	EC0317.22	AA	AA	AA
CHU17	EC0317.23	AA	AA	AA
CHU17	EC0317.24	AA	AA	AA

CHU17	EC0317.25	AA	AA	AA
CHU17	EC0317.26	AA	AA	AA
CHU17	EC0317.27	AA	AA	AA
CHU17	EC0317.28	AA	AA	AA
CHU17	EC0317.29	AA	AA	AA
CHU17	EC0317.30	AA	AA	AA

APPENDIX VIII

CYPRINODON COLLECTION MARCH 2003

Appendix VIII. *Cyprinodon* collection for March 2003

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0331.01	BB	BB	BB
CHU01	EC0331.02	BB	BB	BB
CHU01	EC0331.03	BB	BB	BB
CHU01	EC0331.04	BB	BB	BB
CHU01	EC0331.05	BB	BB	BB
CHU01	EC0331.06	BB	BB	BB
CHU01	EC0331.07	BB	BB	BB
CHU01	EC0331.08	BB	BB	BB
CHU01	EC0331.09	BB	BB	BB
CHU01	EC0331.10	BB	BB	BB
CHU01	EC0331.11	BB	BB	BB
CHU01	EC0331.12	BB	BB	BB
CHU01	EC0331.13	BB	BB	BB
CHU01	EC0331.14	BB	BB	BB
CHU01	EC0331.15	BB	BB	BB
CHU01	EC0331.16	BB	BB	BB
CHU01	EC0331.17	BB	BB	BB
CHU01	EC0331.18	BB	BB	BB
CHU01	EC0331.19	BB	BB	BB
CHU01	EC0331.20	BB	BB	BB
CHU01	EC0331.21	BB	BB	BB
CHU01	EC0331.22	BB	BB	BB
CHU01	EC0331.23	BB	BB	BB
CHU01	EC0331.24	BB	BB	BB
CHU01	EC0331.25	BB	BB	BB
CHU01	EC0331.26	BB	BB	BB
CHU01	EC0331.27	BB	BB	BB
CHU01	EC0331.28	BB	BB	BB
CHU01	EC0331.29	BB	BB	BB
CHU01	EC0331.30	BB	BB	BB
CHU02	EC0332.01	BB	BB	BB
CHU02	EC0332.02	BB	BB	BB
CHU02	EC0332.03	BB	BB	BB
CHU02	EC0332.04	BB	BB	BB
CHU02	EC0332.05	BB	BB	BB
CHU02	EC0332.06	BB	BB	BB
CHU02	EC0332.07	BB	BB	BB
CHU02	EC0332.08	BB	BB	BB

CHU02	EC0332.09	BB	BB	BB
CHU02	EC0332.10	BB	BB	BB
CHU02	EC0332.11	BB	BB	BB
CHU02	EC0332.12	BB	BB	BB
CHU02	EC0332.13	BB	BB	BB
CHU02	EC0332.14	BB	BB	BB
CHU02	EC0332.15	BB	BB	BB
CHU02	EC0332.16	BB	BB	BB
CHU02	EC0332.17	BB	BB	BB
CHU02	EC0332.18	BB	BB	BB
CHU02	EC0332.19	BB	BB	BB
CHU02	EC0332.20	BB	BB	BB
CHU02	EC0332.21	BB	BB	BB
CHU02	EC0332.22	BB	BB	BB
CHU02	EC0332.23	BB	BB	BB
CHU02	EC0332.24	BB	BB	BB
CHU02	EC0332.25	BB	BB	BB
CHU02	EC0332.26	BB	BB	BB
CHU02	EC0332.27	BB	BB	BB
CHU02	EC0332.28	BB	BB	BB
CHU02	EC0332.29	BB	BB	BB
CHU02	EC0332.30	BB	BB	BB
CHU03	EC0333.01	BB	BB	BB
CHU03	EC0333.02	BB	BB	BB
CHU03	EC0333.03	BB	BB	BB
CHU03	EC0333.04	BB	BB	BB
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CHU03	EC0333.07	BB	BB	BB
CHU03	EC0333.08	BB	BB	BB
CHU03	EC0333.09	BB	BB	BB
CHU03	EC0333.10	BB	BB	BB
CHU03	EC0333.11	BB	BB	BB
CHU03	EC0333.12	BB	BB	BB
CHU03	EC0333.13	BB	BB	BB
CHU03	EC0333.14	BB	BB	BB
CHU03	EC0333.15	BB	BB	BB
CHU03	EC0333.16	BB	BB	BB
CHU03	EC0333.17	BB	BB	BB
CHU03	EC0333.18	BB	??	BB
CHU03	EC0333.19	BB	BB	BB
CHU03	EC0333.20	BB	BB	BB
CHU03	EC0333.21	BB	BB	BB

CHU03	EC0333.22	BB	BB	BB
CHU03	EC0333.23	BB	BB	BB
CHU03	EC0333.24	BB	BB	BB
CHU03	EC0333.25	BB	BB	BB
CHU03	EC0333.26	BB	BB	BB
CHU03	EC0333.27	BB	BB	BB
CHU03	EC0333.28	BB	BB	BB
CHU03	EC0333.29	BB	BB	BB
CHU03	EC0333.30	BB	BB	BB
CHU04	EC0334.01	BB	BB	BB
CHU04	EC0334.02	BB	BB	BB
CHU04	EC0334.03	BB	BB	BB
CHU04	EC0334.04	BB	BB	BB
CHU04	EC0334.05	BB	BB	BB
CHU04	EC0334.06	BB	BB	BB
CHU04	EC0334.07	BB	BB	BB
CHU04	EC0334.08	AB	BB	AB
CHU04	EC0334.09	BB	BB	BB
CHU04	EC0334.10	AB	AB	AB
CHU04	EC0334.11	BB	BB	BB
CHU04	EC0334.12	BB	BB	BB
CHU04	EC0334.13	BB	BB	BB
CHU04	EC0334.14	BB	BB	BB
CHU04	EC0334.15	BB	BB	BB
CHU04	EC0334.16	AB	BB	AB
CHU04	EC0334.17	BB	BB	BB
CHU04	EC0334.18	BB	BB	BB
CHU04	EC0334.19	BB	BB	BB
CHU04	EC0334.20	BB	BB	AB
CHU04	EC0334.21	BB	BB	BB
CHU04	EC0334.22	BB	AB	BB
CHU04	EC0334.23	BB	BB	BB
CHU04	EC0334.24	BB	BB	BB
CHU04	EC0334.25	BB	BB	AB
CHU04	EC0334.26	BB	BB	BB
CHU04	EC0334.27	BB	BB	BB
CHU04	EC0334.28	BB	BB	BB
CHU04	EC0334.29	BB	BB	BB
CHU04	EC0334.30	NA	NA	NA
CHU05	EC0335.01	BB	BB	BB
CHU05	EC0335.02	AB	BB	BB

CHU05	EC0335.03	BB	BB	BB
CHU05	EC0335.04	BB	BB	BB
CHU05	EC0335.05	BB	BB	BB
CHU05	EC0335.06	BB	BB	BB
CHU05	EC0335.07	BB	BB	BB
CHU05	EC0335.08	BB	BB	BB
CHU05	EC0335.09	AB	AA	AB
CHU05	EC0335.10	AB	BB	AB
CHU05	EC0335.11	BB	BB	BB
CHU05	EC0335.12	BB	BB	BB
CHU05	EC0335.13	BB	BB	BB
CHU05	EC0335.14	BB	BB	BB
CHU05	EC0335.15	AB	AB	AB
CHU05	EC0335.16	BB	BB	BB
CHU05	EC0335.17	BB	BB	BB
CHU05	EC0335.18	BB	BB	BB
CHU05	EC0335.19	BB	BB	BB
CHU05	EC0335.20	BB	BB	BB
CHU05	EC0335.21	BB	BB	BB
CHU05	EC0335.22	BB	BB	BB
CHU05	EC0335.23	AB	AB	AB
CHU05	EC0335.24	BB	BB	BB
CHU05	EC0335.25	BB	BB	BB
CHU05	EC0335.26	BB	BB	BB
CHU05	EC0335.27	BB	BB	BB
CHU05	EC0335.28	BB	BB	BB
CHU05	EC0335.29	BB	BB	BB
CHU05	EC0335.30	NA	NA	NA
CHU06	EC0336.01	AA	AA	AA
CHU06	EC0336.02	AA	AA	AA
CHU06	EC0336.03	AA	AA	AA
CHU06	EC0336.04	AA	AA	AA
CHU06	EC0336.05	AA	AA	AA
CHU06	EC0336.06	AA	AA	AA
CHU06	EC0336.07	AA	AA	AA
CHU06	EC0336.08	AA	AA	AA
CHU06	EC0336.09	AA	AA	AA
CHU06	EC0336.10	AA	AA	AA
CHU06	EC0336.11	AA	AA	AA
CHU06	EC0336.12	AA	AA	AA
CHU06	EC0336.13	AA	AA	AA
CHU06	EC0336.14	AB	AA	??
CHU06	EC0336.15	AA	AA	AA

CHU06	EC0336.16	AA	AA	AA
CHU06	EC0336.17	AA	AA	AA
CHU06	EC0336.18	AA	AA	AA
CHU06	EC0336.19	AA	AA	AA
CHU06	EC0336.20	AA	AA	AA
CHU06	EC0336.21	AA	AA	AA
CHU06	EC0336.22	AA	AA	AA
CHU06	EC0336.23	AA	AA	AA
CHU06	EC0336.24	AA	AB	AA
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CHU06	EC0336.26	AA	AA	AA
CHU06	EC0336.27	AA	AA	AA
CHU06	EC0336.28	AA	AA	AA
CHU06	EC0336.29	AA	??	AA
CHU06	EC0336.30	AA	AA	AA
CHU07	EC0337.01	AB	AB	BB
CHU07	EC0337.02	AA	AA	AA
CHU07	EC0337.03	AA	AA	AA
CHU07	EC0337.04	BB	BB	BB
CHU07	EC0337.05	AA	AA	AA
CHU07	EC0337.06	AA	AA	AA
CHU07	EC0337.07	AA	AA	AA
CHU07	EC0337.08	AA	AA	AA
CHU07	EC0337.09	AA	AA	AA
CHU07	EC0337.10	AB	BB	BB
CHU07	EC0337.11	AA	AA	AA
CHU07	EC0337.12	AA	AA	AA
CHU07	EC0337.13	AA	AA	AA
CHU07	EC0337.14	AA	AA	AA
CHU07	EC0337.15	??	BB	BB
CHU07	EC0337.16	AB	AB	AB
CHU07	EC0337.17	AA	AA	AA
CHU07	EC0337.18	AA	AA	AA
CHU07	EC0337.19	AA	AA	AA
CHU07	EC0337.20	AA	AB	AA
CHU07	EC0337.21	AB	AB	AB
CHU07	EC0337.22	BB	BB	BB
CHU07	EC0337.23	AB	AB	AB
CHU07	EC0337.24	BB	BB	AB
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CHU07	EC0337.26	AA	AA	AA
CHU07	EC0337.27	AB	AB	AB
CHU07	EC0337.28	BB	BB	BB

CHU07	EC0337.29	BB	BB	BB
CHU07	EC0337.30	AA	AA	AA
CHU08	EC0338.01	AB	BB	AB
CHU08	EC0338.02	BB	BB	AB
CHU08	EC0338.03	AB	AB	AA
CHU08	EC0338.04	AB	AB	BB
CHU08	EC0338.05	AA	AA	AA
CHU08	EC0338.06	AB	BB	AB
CHU08	EC0338.07	AB	BB	BB
CHU08	EC0338.08	AB	AB	BB
CHU08	EC0338.09	AB	AA	AA
CHU08	EC0338.10	AB	??	AB
CHU08	EC0338.11	BB	AB	AB
CHU08	EC0338.12	BB	BB	BB
CHU08	EC0338.13	BB	BB	BB
CHU08	EC0338.14	BB	BB	BB
CHU08	EC0338.15	AA	AB	AA
CHU08	EC0338.16	BB	BB	BB
CHU08	EC0338.17	AA	AA	AA
CHU08	EC0338.18	AA	AA	AA
CHU08	EC0338.19	AB	AB	AB
CHU08	EC0338.20	BB	BB	BB
CHU08	EC0338.21	AB	AB	AA
CHU08	EC0338.22	BB	AB	AB
CHU08	EC0338.23	AB	BB	AB
CHU08	EC0338.24	AA	AA	AB
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CHU08	EC0338.26	AB	AB	AB
CHU08	EC0338.27	AB	AB	AB
CHU08	EC0338.28	AA	AA	AA
CHU08	EC0338.29	BB	BB	BB
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CHU09	EC0339.03	AB	AB	AB
CHU09	EC0339.04	AA	AA	AA
CHU09	EC0339.05	AB	AB	AB
CHU09	EC0339.06	BB	BB	BB
CHU09	EC0339.07	AB	AB	AA
CHU09	EC0339.08	AB	AA	AB
CHU09	EC0339.09	AA	AA	AA

CHU09	EC0339.10	AB	AB	AB
CHU09	EC0339.11	AB	AB	AB
CHU09	EC0339.12	AB	AB	AA
CHU09	EC0339.13	AA	AA	AB
CHU09	EC0339.14	AB	AB	AB
CHU09	EC0339.15	AB	AB	AB
CHU09	EC0339.16	AB	AA	AB
CHU09	EC0339.17	AB	AB	AB
CHU09	EC0339.18	AB	AB	AB
CHU09	EC0339.19	AB	AB	AB
CHU09	EC0339.20	BB	BB	BB
CHU09	EC0339.21	AB	BB	AB
CHU09	EC0339.22	AB	??	AB
CHU09	EC0339.23	AB	BB	AB
CHU09	EC0339.24	AA	AA	AA
CHU09	EC0339.25	AB	AB	AB
CHU09	EC0339.26	AB	AB	BB
CHU09	EC0339.27	AA	AA	AA
CHU09	EC0339.28	AB	AB	BB
CHU09	EC0339.29	AA	AA	AB
CHU09	EC0339.30	NA	NA	NA
CHU10	EC0340.01	AA	AA	AA
CHU10	EC0340.02	AB	AB	AB
CHU10	EC0340.03	AA	AA	AA
CHU10	EC0340.04	AA	AA	AA
CHU10	EC0340.05	AA	AA	AA
CHU10	EC0340.06	AB	AB	AA
CHU10	EC0340.07	AB	AA	AB
CHU10	EC0340.08	AA	AA	AA
CHU10	EC0340.09	AB	BB	AB
CHU10	EC0340.10	AA	AA	AA
CHU10	EC0340.11	AA	AA	AA
CHU10	EC0340.12	AA	AA	AB
CHU10	EC0340.13	AA	AA	AA
CHU10	EC0340.14	AA	AA	AA
CHU10	EC0340.15	AA	AA	AA
CHU10	EC0340.16	AA	AA	AA
CHU10	EC0340.17	AA	??	AA
CHU10	EC0340.18	AB	AA	AA
CHU10	EC0340.19	AA	AA	AA
CHU10	EC0340.20	AA	AA	AA
CHU10	EC0340.21	AA	AA	AA
CHU10	EC0340.22	AA	AB	BB

CHU10	EC0340.23	AB	AB	AA
CHU10	EC0340.24	AB	AA	AB
CHU10	EC0340.25	AA	AA	AA
CHU10	EC0340.26	AA	AA	AA
CHU10	EC0340.27	BB	AB	AB
CHU10	EC0340.28	AA	AA	AB
CHU10	EC0340.29	AA	AA	AA
CHU10	EC0340.30	AB	AB	AB
CHU11	EC0341.01	AB	AB	AB
CHU11	EC0341.02	AA	AA	AA
CHU11	EC0341.03	AA	AA	AA
CHU11	EC0341.04	AA	AA	AA
CHU11	EC0341.05	AA	AB	AA
CHU11	EC0341.06	AB	AB	AB
CHU11	EC0341.07	AA	AA	AA
CHU11	EC0341.08	AA	AA	AA
CHU11	EC0341.09	AB	AB	AB
CHU11	EC0341.10	AB	AB	AB
CHU11	EC0341.11	AA	BB	AB
CHU11	EC0341.12	NA	NA	NA
CHU11	EC0341.13	NA	NA	NA
CHU11	EC0341.14	NA	NA	NA
CHU11	EC0341.15	NA	NA	NA
CHU11	EC0341.16	NA	NA	NA
CHU11	EC0341.17	NA	NA	NA
CHU11	EC0341.18	NA	NA	NA
CHU11	EC0341.19	NA	NA	NA
CHU11	EC0341.20	NA	NA	NA
CHU11	EC0341.21	NA	NA	NA
CHU11	EC0341.22	NA	NA	NA
CHU11	EC0341.23	NA	NA	NA
CHU11	EC0341.24	NA	NA	NA
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CHU11	EC0341.28	NA	NA	NA
CHU11	EC0341.29	NA	NA	NA
CHU11	EC0341.30	NA	NA	NA
CHU12	EC0342.01	AA	AA	AA
CHU12	EC0342.02	AA	AA	AA
CHU12	EC0342.03	AA	AA	AA

CHU12	EC0342.04	AA	AA	AA
CHU12	EC0342.05	AA	AA	AA
CHU12	EC0342.06	AB	AA	AA
CHU12	EC0342.07	AA	AA	AA
CHU12	EC0342.08	AA	AA	AA
CHU12	EC0342.09	AA	AA	AA
CHU12	EC0342.10	AB	AA	AA
CHU12	EC0342.11	AA	AA	AA
CHU12	EC0342.12	AA	AA	AA
CHU12	EC0342.13	AA	AA	AA
CHU12	EC0342.14	AA	AA	AA
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CHU12	EC0342.16	AA	??	AA
CHU12	EC0342.17	AA	AA	AA
CHU12	EC0342.18	AA	AA	AA
CHU12	EC0342.19	AA	AB	AA
CHU12	EC0342.20	AA	AB	AA
CHU12	EC0342.21	AA	AA	AA
CHU12	EC0342.22	AA	AB	AA
CHU12	EC0342.23	AA	AA	AA
CHU12	EC0342.24	AA	AA	AA
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CHU12	EC0342.29	AA	AB	AA
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CHU13	EC0343.02	AA	AA	AA
CHU13	EC0343.03	AA	AA	AB
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CHU13	EC0343.05	AA	AA	AA
CHU13	EC0343.06	AA	AA	AA
CHU13	EC0343.07	AA	AA	AA
CHU13	EC0343.08	AA	AA	AA
CHU13	EC0343.09	AA	AA	AA
CHU13	EC0343.10	AA	AA	AA
CHU13	EC0343.11	AA	AA	AA
CHU13	EC0343.12	AA	AA	AA
CHU13	EC0343.13	AA	AA	AA
CHU13	EC0343.14	AA	AA	AA
CHU13	EC0343.15	AA	AA	AA
CHU13	EC0343.16	AA	AA	AA

CHU13	EC0343.17	AA	??	AA
CHU13	EC0343.18	AA	AA	AA
CHU13	EC0343.19	AA	AA	AA
CHU13	EC0343.20	AA	AA	AA
CHU13	EC0343.21	AA	AA	AA
CHU13	EC0343.22	AA	AA	AA
CHU13	EC0343.23	AA	??	AB
CHU13	EC0343.24	AB	AA	AB
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CHU13	EC0343.27	AA	AB	AA
CHU13	EC0343.28	AA	AB	AA
CHU13	EC0343.29	AA	AA	AB
CHU13	EC0343.30	BB	AB	AA

CHU14	EC0344.01	AA	AB	AB
CHU14	EC0344.02	AA	AA	AA
CHU14	EC0344.03	AA	AB	AB
CHU14	EC0344.04	AA	AA	AA
CHU14	EC0344.05	AA	AA	AA
CHU14	EC0344.06	AA	AA	AB
CHU14	EC0344.07	AB	BB	BB
CHU14	EC0344.08	AA	AA	AA
CHU14	EC0344.09	AA	AA	AA
CHU14	EC0344.10	AA	AA	AA
CHU14	EC0344.11	AA	AA	AA
CHU14	EC0344.12	AA	AA	AA
CHU14	EC0344.13	AA	AB	AA
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CHU14	EC0344.17	AA	AA	AA
CHU14	EC0344.18	AA	AA	AA
CHU14	EC0344.19	AA	AA	AA
CHU14	EC0344.20	AA	AA	AA
CHU14	EC0344.21	AA	AA	AA
CHU14	EC0344.22	AA	AA	AA
CHU14	EC0344.23	AA	AA	AA
CHU14	EC0344.24	AA	AA	AA
CHU14	EC0344.25	AA	AA	AA
CHU14	EC0344.26	AA	AB	AA
CHU14	EC0344.27	AA	AB?	AB
CHU14	EC0344.28	BB	AA	AA
CHU14	EC0344.29	AB	AB	AB

CHU14	EC0344.30	AB	AA	AB
CHU15	EC0345.01	AA	AA	AA
CHU15	EC0345.02	AA	AA	AA
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CHU15	EC0345.05	AA	AA	AB
CHU15	EC0345.06	AA	AA	AA
CHU15	EC0345.07	AA	AA	AA
CHU15	EC0345.08	AA	AA	AA
CHU15	EC0345.09	AA	AA	AA
CHU15	EC0345.10	AA	AA	AA
CHU15	EC0345.11	AA	AA	AA
CHU15	EC0345.12	AA	AA	AA
CHU15	EC0345.13	AA	AA	AA
CHU15	EC0345.14	AA	AA	AB
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CHU15	EC0345.16	AA	AA	AA
CHU15	EC0345.17	AA	AA	AA
CHU15	EC0345.18	AA	AA	AA
CHU15	EC0345.19	AA	AA	AA
CHU15	EC0345.20	AB	AA	AA
CHU15	EC0345.21	AB	BB	AB
CHU15	EC0345.22	AA	AA	AB
CHU15	EC0345.23	AA	AA	AA
CHU15	EC0345.24	AA	AA	AA
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CHU15	EC0345.26	AA	AA	AA
CHU15	EC0345.27	AA	AA	AA
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CHU15	EC0345.29	AB	AA	AB
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CHU16	EC0346.03	AA	AA	AB
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CHU16	EC0346.05	AA	AA	AA
CHU16	EC0346.06	AA	AA	AA
CHU16	EC0346.07	AA	AA	AA
CHU16	EC0346.08	AA	AA	AB
CHU16	EC0346.09	AA	AA	AA
CHU16	EC0346.10	AA	AA	AA

CHU16	EC0346.11	AA	AA	AA
CHU16	EC0346.12	AA	AA	AB
CHU16	EC0346.13	AA	AA	AA
CHU16	EC0346.14	AA	AA	AA
CHU16	EC0346.15	AA	AA	AA
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CHU16	EC0346.17	AA	AA	AA
CHU16	EC0346.18	AA	AA	AB
CHU16	EC0346.19	AA	AA	AB
CHU16	EC0346.20	AA	AA	AA
CHU16	EC0346.21	AA	AA	AA
CHU16	EC0346.22	AA	AA	AA
CHU16	EC0346.23	AA	AA	AA
CHU16	EC0346.24	AA	AA	AA
CHU16	EC0346.25	AA	AA	AA
CHU16	EC0346.26	AA	AA	AA
CHU16	EC0346.27	AA	AA	??
CHU16	EC0346.28	AA	AA	AA
CHU16	EC0346.29	AA	AA	AA
CHU16	EC0346.30	AA	AA	AA
CHU17	EC0347.01	AA	AA	AA
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CHU17	EC0347.04	AA	AA	AA
CHU17	EC0347.05	AA	AA	AA
CHU17	EC0347.06	AA	AA	AA
CHU17	EC0347.07	AA	AA	AA
CHU17	EC0347.08	AA	AA	AA
CHU17	EC0347.09	AA	AA	AA
CHU17	EC0347.10	AA	AA	AA
CHU17	EC0347.11	AA	AA	AA
CHU17	EC0347.12	AA	AA	AA
CHU17	EC0347.13	AA	AA	AA
CHU17	EC0347.14	AA	AA	AA
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CHU17	EC0347.16	AA	AA	AA
CHU17	EC0347.17	AA	AA	AA
CHU17	EC0347.18	AA	AA	AA
CHU17	EC0347.19	AA	AA	AA
CHU17	EC0347.20	AA	AA	AA
CHU17	EC0347.21	AA	AA	AB
CHU17	EC0347.22	??	AA	??
CHU17	EC0347.23	AA	AA	AB

CHU17	EC0347.24	AA	AA	AA
CHU17	EC0347.25	AA	AA	AA
CHU17	EC0347.26	AA	AA	AA
CHU17	EC0347.27	AA	AA	AA
CHU17	EC0347.28	AA	AA	AA
CHU17	EC0347.29	AA	AA	AB
CHU17	EC0347.30	AA	AA	AA

APPENDIX IX.

CYPRINODON COLLECTION FOR AUGUST 2003

Appendix IX. *Cyprinodon* collection for August 2003

Site #	Collect #	CK	RAG	TPI-B
CHU01	EC0361.01	BB	BB	BB
CHU01	EC0361.02	BB	BB	BB
CHU01	EC0361.03	BB	BB	BB
CHU01	EC0361.04	BB	BB	BB
CHU01	EC0361.05	BB	BB	BB
CHU01	EC0361.06	BB	BB	BB
CHU01	EC0361.07	BB	BB	BB
CHU01	EC0361.08	BB	BB	BB
CHU01	EC0361.09	BB	BB	BB
CHU01	EC0361.10	BB	BB	BB
CHU01	EC0361.11	BB	BB	BB
CHU01	EC0361.12	BB	BB	BB
CHU01	EC0361.13	BB	BB	BB
CHU01	EC0361.14	BB	BB	BB
CHU01	EC0361.15	BB	BB	BB
CHU01	EC0361.16	BB	BB	BB
CHU01	EC0361.17	BB	BB	BB
CHU01	EC0361.18	BB	BB	BB
CHU01	EC0361.19	BB	BB	BB
CHU01	EC0361.20	BB	BB	BB
CHU01	EC0361.21	BB	BB	BB
CHU01	EC0361.22	BB	BB	BB
CHU01	EC0361.23	BB	BB	BB
CHU01	EC0361.24	BB	BB	BB
CHU01	EC0361.25	BB	BB	BB
CHU01	EC0361.26	BB	BB	BB
CHU01	EC0361.27	BB	BB	BB
CHU01	EC0361.28	BB	BB	BB
CHU01	EC0361.29	BB	BB	BB
CHU01	EC0361.30	BB	BB	BB
CHU02	EC0362.01	BB	BB	BB
CHU02	EC0362.02	BB	BB	BB
CHU02	EC0362.03	BB	BB	BB
CHU02	EC0362.04	BB	BB	BB
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CHU02	EC0362.06	BB	BB	BB
CHU02	EC0362.07	BB	BB	BB

CHU02	EC0362.08	BB	BB	BB
CHU02	EC0362.09	BB	BB	BB
CHU02	EC0362.10	BB	BB	BB
CHU02	EC0362.11	BB	BB	BB
CHU02	EC0362.12	BB	BB	BB
CHU02	EC0362.13	NA	NA	NA
CHU02	EC0362.14	NA	NA	NA
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CHU02	EC0362.17	NA	NA	NA
CHU02	EC0362.18	NA	NA	NA
CHU02	EC0362.19	NA	NA	NA
CHU02	EC0362.20	NA	NA	NA
CHU02	EC0362.21	NA	NA	NA
CHU02	EC0362.22	NA	NA	NA
CHU02	EC0362.23	NA	NA	NA
CHU02	EC0362.24	NA	NA	NA
CHU02	EC0362.25	NA	NA	NA
CHU02	EC0362.26	NA	NA	NA
CHU02	EC0362.27	NA	NA	NA
CHU02	EC0362.28	NA	NA	NA
CHU02	EC0362.29	NA	NA	NA
CHU02	EC0362.30	NA	NA	NA
CHU03	EC0363.01	BB	BB	BB
CHU03	EC0363.02	BB	BB	BB
CHU03	EC0363.03	BB	BB	BB
CHU03	EC0363.04	BB	BB	BB
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CHU03	EC0363.06	BB	BB	BB
CHU03	EC0363.07	BB	BB	BB
CHU03	EC0363.08	BB	BB	BB
CHU03	EC0363.09	BB	BB	BB
CHU03	EC0363.10	BB	BB	BB
CHU03	EC0363.11	BB	BB	BB
CHU03	EC0363.12	BB	BB	BB
CHU03	EC0363.13	BB	BB	BB
CHU03	EC0363.14	BB	BB	BB
CHU03	EC0363.15	BB	BB	BB
CHU03	EC0363.16	BB	BB	BB
CHU03	EC0363.17	BB	BB	BB
CHU03	EC0363.18	BB	BB	BB
CHU03	EC0363.19	BB	BB	BB

CHU03	EC0363.20	BB	BB	BB
CHU03	EC0363.21	BB	BB	BB
CHU03	EC0363.22	BB	BB	BB
CHU03	EC0363.23	BB	BB	BB
CHU03	EC0363.24	BB	BB	BB
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CHU03	EC0363.26	BB	BB	BB
CHU03	EC0363.27	AA	AA	AA
CHU03	EC0363.28	BB	BB	BB
CHU03	EC0363.29	BB	BB	BB
CHU03	EC0363.30	NA	NA	NA
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CHU04	EC0364.02	BB	BB	BB
CHU04	EC0364.03	BB	BB	BB
CHU04	EC0364.04	BB	BB	BB
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CHU04	EC0364.07	BB	BB	BB
CHU04	EC0364.08	BB	BB	BB
CHU04	EC0364.09	BB	BB	BB
CHU04	EC0364.10	BB	BB	BB
CHU04	EC0364.11	??	??	BB
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CHU04	EC0364.13	BB	BB	BB
CHU04	EC0364.14	BB	BB	BB
CHU04	EC0364.15	BB	BB	BB
CHU04	EC0364.16	BB	BB	BB
CHU04	EC0364.17	BB	BB	BB
CHU04	EC0364.18	BB	BB	BB
CHU04	EC0364.19	BB	BB	BB
CHU04	EC0364.20	AB	BB	BB
CHU04	EC0364.21	BB	BB	BB
CHU04	EC0364.22	BB	BB	BB
CHU04	EC0364.23	BB	??	BB
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CHU04	EC0364.27	BB	BB	BB
CHU04	EC0364.28	BB	BB	BB
CHU04	EC0364.29	BB	BB	BB
CHU04	EC0364.30	BB	??	BB

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CHU05	EC0365.03	AA	AA	AA
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CHU05	EC0365.05	AB	BB	BB
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CHU05	EC0365.07	AB	BB	BB
CHU05	EC0365.08	BB	BB	BB
CHU05	EC0365.09	BB	BB	BB
CHU05	EC0365.10	BB	BB	BB
CHU05	EC0365.11	BB	BB	AB
CHU05	EC0365.12	AA	AA	AA
CHU05	EC0365.13	BB	BB	BB
CHU05	EC0365.14	AB	AB	AB
CHU05	EC0365.15	BB	BB	BB
CHU05	EC0365.16	BB	BB	BB
CHU05	EC0365.17	BB	BB	AB
CHU05	EC0365.18	BB	BB	BB
CHU05	EC0365.19	BB	BB	BB
CHU05	EC0365.20	BB	BB	BB
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CHU05	EC0365.22	BB	BB	BB
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CHU05	EC0365.27	BB	BB	BB
CHU05	EC0365.28	AA	AB	AB
CHU05	EC0365.29	??	AA	AA
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CHU06	EC0366.04	AA	AA	AA
CHU06	EC0366.05	AA	AA	AA
CHU06	EC0366.06	AA	AA	AA
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CHU06	EC0366.08	AA	AA	AA
CHU06	EC0366.09	AA	AA	AA
CHU06	EC0366.10	AA	AA	AA
CHU06	EC0366.11	AA	AA	AA

CHU06	EC0366.12	AA	AA	AA
CHU06	EC0366.13	AA	AA	AA
CHU06	EC0366.14	AA	AA	AA
CHU06	EC0366.15	AA	AA	AA
CHU06	EC0366.16	AA	AA	AA
CHU06	EC0366.17	AA	AA	AA
CHU06	EC0366.18	AA	AA	AA
CHU06	EC0366.19	AA	AA	AA
CHU06	EC0366.20	AA	AA	AA
CHU06	EC0366.21	AA	AA	AA
CHU06	EC0366.22	AA	AA	AA
CHU06	EC0366.23	AA	AA	AA
CHU06	EC0366.24	AA	AA	AA
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CHU07	EC0367.03	AA	AA	AA
CHU07	EC0367.04	AA	AA	AA
CHU07	EC0367.05	AA	AA	??
CHU07	EC0367.06	AA	AA	AA
CHU07	EC0367.07	AA	AA	AA
CHU07	EC0367.08	AA	AA	AA
CHU07	EC0367.09	AA	AA	AA
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CHU07	EC0367.17	AA	AA	AA
CHU07	EC0367.18	AA	AA	??
CHU07	EC0367.19	AA	AA	AA
CHU07	EC0367.20	AA	AA	AA
CHU07	EC0367.21	AA	AA	AA
CHU07	EC0367.22	AA	AA	AA
CHU07	EC0367.23	AA	AA	AA

CHU07	EC0367.24	AA	AA	AA
CHU07	EC0367.25	AA	AA	AA
CHU07	EC0367.26	AA	AA	AA
CHU07	EC0367.27	AA	AA	AA
CHU07	EC0367.28	AA	AA	AA
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CHU08	EC0368.03	AA	AA	AB
CHU08	EC0368.04	AA	AA	AA
CHU08	EC0368.05	AA	AA	AA
CHU08	EC0368.06	AA	AA	AA
CHU08	EC0368.07	AA	AA	??
CHU08	EC0368.08	AA	AA	AA
CHU08	EC0368.09	AA	AA	AA
CHU08	EC0368.10	AA	AA	AA
CHU08	EC0368.11	AB	AB	AB
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CHU08	EC0368.13	AA	AA	AA
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CHU08	EC0368.17	AA	AA	AA
CHU08	EC0368.18	AA	AA	AA
CHU08	EC0368.19	AA	AA	AA
CHU08	EC0368.20	AB	AB	AA
CHU08	EC0368.21	AA	AB	AA
CHU08	EC0368.22	AA	AA	AA
CHU08	EC0368.23	AA	AA	AA
CHU08	EC0368.24	AA	AA	AA
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CHU08	EC0368.30	AA	AA	AB
CHU09	EC0369.01	BB	BB	BB
CHU09	EC0369.02	AA	AA	AA
CHU09	EC0369.03	AB	AA	AA

CHU09	EC0369.04	AB	AB	AB
CHU09	EC0369.05	AB	AB	BB
CHU09	EC0369.06	AB	AB	AB
CHU09	EC0369.07	AB	AB	AB
CHU09	EC0369.08	AB	BB	BB
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CHU09	EC0369.17	AA	AB	AB
CHU09	EC0369.18	AB	BB	BB
CHU09	EC0369.19	AB	AB	AB
CHU09	EC0369.20	BB	AB	BB
CHU09	EC0369.21	AB	BB	BB
CHU09	EC0369.22	AB	AB	AB
CHU09	EC0369.23	BB	BB	BB
CHU09	EC0369.24	BB	AB	AB
CHU09	EC0369.25	AB	AB	AB
CHU09	EC0369.26	BB	BB	BB
CHU09	EC0369.27	AA	AB	AA
CHU09	EC0369.28	AA	BB	AB
CHU09	EC0369.29	AB	AB	AB
CHU09	EC0369.30	AB	AB	BB
CHU10	EC0370.01	AA	AB	AA
CHU10	EC0370.02	BB	BB	AB
CHU10	EC0370.03	AA	AA	AA
CHU10	EC0370.04	AA	AA	AB
CHU10	EC0370.05	AA	AA	AA
CHU10	EC0370.06	AB	AA	AB
CHU10	EC0370.07	BB	BB	AB
CHU10	EC0370.08	AB	AB	AB
CHU10	EC0370.09	AB	AB	AB
CHU10	EC0370.10	AB	AA	AB
CHU10	EC0370.11	AB	AB	AA
CHU10	EC0370.12	AA	AB	AB
CHU10	EC0370.13	BB	BB	BB
CHU10	EC0370.14	BB	AB	AB
CHU10	EC0370.15	AA	BB	AB

CHU10	EC0370.16	AA	AA	AA
CHU10	EC0370.17	AB	AB	AB
CHU10	EC0370.18	BB	BB	BB
CHU10	EC0370.19	BB	BB	AB
CHU10	EC0370.20	AB	AB	AB
CHU10	EC0370.21	AB	AB	BB
CHU10	EC0370.22	AA	AB	AB
CHU10	EC0370.23	AA	AB	AB
CHU10	EC0370.24	AB	AB	AA
CHU10	EC0370.25	AA	AA	AA
CHU10	EC0370.26	AB	AB	AB
CHU10	EC0370.27	AA	AA	AA
CHU10	EC0370.28	AB	AB	AB
CHU10	EC0370.29	AA	BB	BB
CHU10	EC0370.30	AB	AA	AB
CHU11	EC0371.01	BB	AB	AB
CHU11	EC0371.02	AA	AA	AB
CHU11	EC0371.03	BB	BB	BB
CHU11	EC0371.04	AB	BB	BB
CHU11	EC0371.05	AB	AB	AA
CHU11	EC0371.06	AB	AB	AA
CHU11	EC0371.07	AA	AA	AA
CHU11	EC0371.08	AB	AB	AB
CHU11	EC0371.09	AA	AB	AB
CHU11	EC0371.10	AA	AB	AB
CHU11	EC0371.11	AA	AB	AB
CHU11	EC0371.12	AA	AB	AA
CHU11	EC0371.13	AB	AA	AB
CHU11	EC0371.14	AA	AA	AB
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CHU11	EC0371.16	AA	AA	AA
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CHU11	EC0371.18	AB	AB	AA
CHU11	EC0371.19	AA	AA	AA
CHU11	EC0371.20	BB	BB	BB
CHU11	EC0371.21	AA	AB	AB
CHU11	EC0371.22	AA	AA	AB
CHU11	EC0371.23	AA	AB	AA
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CHU11	EC0371.25	BB	AB	AA
CHU11	EC0371.26	AB	AB	AB
CHU11	EC0371.27	AB	AB	AB

CHU11	EC0371.28	AA	AA	AA
CHU11	EC0371.29	AA	AA	AA
CHU11	EC0371.30	AA	AB	AB
CHU12	EC0372.01	AA	AA	AA
CHU12	EC0372.02	AB	AB	AB
CHU12	EC0372.03	AA	AA	AA
CHU12	EC0372.04	AA	AB	AA
CHU12	EC0372.05	AA	AA	AA
CHU12	EC0372.06	AB	AA	AA
CHU12	EC0372.07	AA	AB	AA
CHU12	EC0372.08	AA	AB	AA
CHU12	EC0372.09	AA	AA	AA
CHU12	EC0372.10	AA	AA	AA
CHU12	EC0372.11	AA	AA	AA
CHU12	EC0372.12	AB	AB	AA
CHU12	EC0372.13	AB	AB	AA
CHU12	EC0372.14	AA	AA	AA
CHU12	EC0372.15	AB	AB	AA
CHU12	EC0372.16	AA	AB	AA
CHU12	EC0372.17	AA	AA	AA
CHU12	EC0372.18	AA	AA	AA
CHU12	EC0372.19	AA	AA	AA
CHU12	EC0372.20	AA	BB	AA
CHU12	EC0372.21	AA	AA	AA
CHU12	EC0372.22	AA	AA	AA
CHU12	EC0372.23	AA	AA	AA
CHU12	EC0372.24	AA	AA	AA
CHU12	EC0372.25	AA	AA	AA
CHU12	EC0372.26	AA	AA	AA
CHU12	EC0372.27	AA	AA	AA
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CHU12	EC0372.29	AA	AA	AA
CHU12	EC0372.30	AA	AB	AA
CHU13	EC0373.01	AB	AB	BB
CHU13	EC0373.02	AA	AA	AA
CHU13	EC0373.03	AA	AA	AA
CHU13	EC0373.04	AA	AA	AA
CHU13	EC0373.05	AA	AA	AA
CHU13	EC0373.06	AA	AA	AA
CHU13	EC0373.07	AA	AA	AA

CHU13	EC0373.08	AA	AA	AA
CHU13	EC0373.09	AA	AA	AA
CHU13	EC0373.10	AA	AA	AA
CHU13	EC0373.11	AA	AA	AA
CHU13	EC0373.12	AA	AA	AA
CHU13	EC0373.13	AA	AA	AA
CHU13	EC0373.14	AA	AA	AA
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CHU13	EC0373.17	AA	AA	AA
CHU13	EC0373.18	AB	BB	AB
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CHU13	EC0373.24	AA	AA	AA
CHU13	EC0373.25	AA	AB	AA
CHU13	EC0373.26	AA	AA	AB
CHU13	EC0373.27	AA	AB	AA
CHU13	EC0373.28	AB	AB	AA
CHU13	EC0373.29	BB	AB	BB
CHU13	EC0373.30	AA	AB	AB

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CHU14	EC0374.02	AA	AA	AA
CHU14	EC0374.03	AA	AA	AA
CHU14	EC0374.04	AA	AA	AA
CHU14	EC0374.05	AB	AA	AB
CHU14	EC0374.06	AA	AA	AA
CHU14	EC0374.07	AA	AA	AA
CHU14	EC0374.08	AA	AA	AA
CHU14	EC0374.09	AA	AA	AA
CHU14	EC0374.10	AA	AB	AA
CHU14	EC0374.11	AA	AA	AA
CHU14	EC0374.12	AA	AA	AA
CHU14	EC0374.13	AA	AA	AA
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CHU14	EC0374.15	AA	AA	AA
CHU14	EC0374.16	AA	AA	AA
CHU14	EC0374.17	AA	AA	AA
CHU14	EC0374.18	AA	AA	AA
CHU14	EC0374.19	AA	AA	AA

CHU14	EC0374.20	AA	AA	AA
CHU14	EC0374.21	AA	AA	AA
CHU14	EC0374.22	AA	AA	AA
CHU14	EC0374.23	AA	AA	AA
CHU14	EC0374.24	AA	AA	AA
CHU14	EC0374.25	AB	AA	BB
CHU14	EC0374.26	AA	AA	AA
CHU14	EC0374.27	AA	AA	AA
CHU14	EC0374.28	AA	AA	AA
CHU14	EC0374.29	AA	AA	AA
CHU14	EC0374.30	AA	AA	AB
CHU15	EC0375.01	AB	AA	AB
CHU15	EC0375.02	AB	AB	BB
CHU15	EC0375.03	AA	AA	AA
CHU15	EC0375.04	AA	AA	AA
CHU15	EC0375.05	AA	AA	AA
CHU15	EC0375.06	AA	AA	AA
CHU15	EC0375.07	AA	AA	AA
CHU15	EC0375.08	AA	AA	AA
CHU15	EC0375.09	AA	AA	AA
CHU15	EC0375.10	AA	AA	AA
CHU15	EC0375.11	AA	AA	AA
CHU15	EC0375.12	AA	AA	AA
CHU15	EC0375.13	AA	AA	AA
CHU15	EC0375.14	AA	AA	AA
CHU15	EC0375.15	AA	AA	AA
CHU15	EC0375.16	AA	AB	AB
CHU15	EC0375.17	AA	AA	AA
CHU15	EC0375.18	AA	AA	AA
CHU15	EC0375.19	AA	AA	AA
CHU15	EC0375.20	AA	AA	AA
CHU15	EC0375.21	AA	AA	AA
CHU15	EC0375.22	AA	AA	AA
CHU15	EC0375.23	AA	AA	AA
CHU15	EC0375.24	AA	AA	AA
CHU15	EC0375.25	AA	AA	AA
CHU15	EC0375.26	AB	??	AA
CHU15	EC0375.27	AB	AB	AA
CHU15	EC0375.28	AA	AB	AA
CHU15	EC0375.29	AA	??	AA
CHU15	EC0375.30	AA	AA	AA

CHU16	EC0376.01	AA	AA	AA
CHU16	EC0376.02	AA	AA	AA
CHU16	EC0376.03	AA	AA	AA
CHU16	EC0376.04	AA	AA	AB?
CHU16	EC0376.05	AA	AA	AA
CHU16	EC0376.06	AA	AA	AA
CHU16	EC0376.07	AA	AA	AA
CHU16	EC0376.08	AA	AA	AA
CHU16	EC0376.09	AA	AA	AA
CHU16	EC0376.10	AA	AA	AA
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CHU16	EC0376.16	AA	AA	AA
CHU16	EC0376.17	AA	AA	AB?
CHU16	EC0376.18	AA	AA	AA
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CHU16	EC0376.22	AA	AA	AA
CHU16	EC0376.23	AA	AA	AA
CHU16	EC0376.24	AA	AA	AA
CHU16	EC0376.25	AA	AA	AA
CHU16	EC0376.26	AA	AA	AA
CHU16	EC0376.27	AA	AA	AA
CHU16	EC0376.28	AA	AA	AA
CHU16	EC0376.29	AA	AA	AA
CHU16	EC0376.30	AA	AB	AA
CHU17	EC0377.01	AA	AA	AA
CHU17	EC0377.02	AA	AA	AA
CHU17	EC0377.03	AA	AA	AA
CHU17	EC0377.04	AA	AA	AA
CHU17	EC0377.05	AA	AA	AA
CHU17	EC0377.06	AA	AA	AB?
CHU17	EC0377.07	AA	AA	AA
CHU17	EC0377.08	AA	AA	AA
CHU17	EC0377.09	AA	AA	AA
CHU17	EC0377.10	AA	AA	AA
CHU17	EC0377.11	AA	AA	AA

CHU17	EC0377.12	AA	AA	AA
CHU17	EC0377.13	AA	AA	AA
CHU17	EC0377.14	AA	AA	AA
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CHU17	EC0377.16	AA	AA	AA
CHU17	EC0377.17	AA	AA	AA
CHU17	EC0377.18	AA	AA	AA
CHU17	EC0377.19	AA	AA	AA
CHU17	EC0377.20	AA	AA	AA
CHU17	EC0377.21	AA	AA	AA
CHU17	EC0377.22	AA	AA	AA
CHU17	EC0377.23	AA	AA	AB
CHU17	EC0377.24	AA	AA	AA
CHU17	EC0377.25	AA	AA	AA
CHU17	EC0377.26	AA	AA	AA
CHU17	EC0377.27	AA	AA	AA
CHU17	EC0377.28	AA	AA	AA
CHU17	EC0377.29	AA	AA	AA
CHU17	EC0377.30	AA	AA	AA

APPENDIX X.

NUCLEAR GENE SEQUENCES

Appendix X. Nuclear gene sequences for *Cyprinodon atrorus* and *C. bifasciatus* at CK-A7, RAG-1, and TPI-B4

Cyprinodon atrorus-specific alleles

CK-A7. Allele cA1

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTTCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAACGCTGACCGTCTGGGCTCCTCCGAGGT
GGAGCAGGTGCAGCTGGTCGTCGACGG

RAG-1 Allele rA1

GTTTCAGCACGTCTTCCAGCTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC
CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCGCTGGCCCAGGC
TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGCGCAAGGGTCTTGT
GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAAGGTAGAGGAGAGC
AGGTCAGCAAAACGTTGTGAGTTAAAACTGTAGCGGCACAGCTGGTCAGGGC
ACTCCTTGGCT

TPI-B4 Allele tA1

TGAGAGGGAGGGAGGCATCACGGAGAAGGTGGTGTTTGCTCAGACCAAAGT
CATTGCAGGTATGCAGCTTTCAGCAGGGAAGCTGAGTGATGCAACTCCAATT
CGGCTTTGGGGGTGAAAATAAATCTGCTTGTACAATACCAAGTCTTATCTCAT
TGATAGACAATGTAAAG

Cyprinodon bifasciatus-specific alleles

CK-A7 Allele cB1

CCCAAGTTTGAGGAGATCCTGACCAGACTGCGCCTGCAGAAACGTGGCACAG
GTACAGAAACGGTTTCCTTCCTCCTGCCAGGCGGAGAGAACCAGGATCTGAA
CTGGGACTCATTCACTCTCTTTCTCCTTCCAGGCGGTGTGGACACAGCCTCAG
TGGGCGGCGTGTTCGACATCTCCAATGCTGACCGTCTGGGCTCCTCCGAGGTG
GAGCAGGTGCAGCTGGTCGTCGACGG

RAG-1 Allele rB1

GTTTCAGCACGTCTTCCAGTTCAAAGGCCTTTGACTGCCGTGCATTCATCTTC
CTGAAACGCCTAAACAGTTTATTTGCTGACTCATTCCCCTCGCTGGCCCAAGC
TCCTATGGATCCATCTCTCTCTATTATTTCAGGCACATGTGCAAGGGTCTTGT
GCAGATAGTTGGTTATTTTTCCATTGTATCTGTACTTGAAGGTAGAGGAGAGC
AGGTCAGCAAAACGTTGTGARTTAAAACTGTAGCGGCACAGCTGGTCAGGGC
ACTCCTTGGCT

TPI-B4 Allele tB1
TGAGAGGGAGGGAGGCATCACGGAGAAGGTGGTGTTTGCTCAGACCAAAGT
CATCGCAGGTATGCAGCTTTCAGCAGGGAAGCTGAGTGATGCAACTCCATTT
CGGCTTTGGGGGTGAAAATAAATCTGCTTGTACAATACCAAGTCTTATCTCAT
TGATAGACAATGTAAAG

CHAPTER 5

Synthesis

Historically, there has been considerable controversy over the evolutionary importance of hybridization. Botanists have long regarded hybridization as a pervasive and creative evolutionary force (Lotsy 1916, Anderson 1948, 1949, Anderson and Stebbins 1954, Arnold 1992, Reiseberg 1993; but see Heiser 1949), whereas zoologists have generally considered hybridization an evolutionary dead end (Fisher 1930, Mayr 1963, Barton 1979, 1980, 1983, Barton and Hewitt 1985, 1989, Hewitt 1988; but see Dobzhansky 1941). Specifically, animal hybrid zones have most often been classified as "tension zones," where hybrid zone dynamics are regulated by a balance between selection against hybrids and dispersal of parental species into the hybrid zone (Barton and Hewitt 1985). The tension zone model assumes hybrids are unfit regardless of environmental circumstances. Accumulating evidence, however, suggests that environment can play a critical, governing role in animal as well as plant hybrid zones, even in hybrid zones that fit many tension zone expectations (reviewed in Arnold 1997).

While there is growing evidence that environmental variation may play an important role in regulation of hybridization in plant and animal systems, there has been only limited investigation into the role of environment in shaping hybrid zone dynamics. Importantly, there has been a dearth of studies that address spatio-temporal dynamics within both plant and animal hybrid zones. This study sought to determine if seasonal and inter-annual environmental variation had significant influences on the regulation of hybridization between two pupfish species, *Cyprinodon atrorus* and *C. bifasciatus*, endemic to Cuatro Cienegas, Mexico. This system presents an excellent model for

investigating the role of exogenous selection in animal hybrid zones, as these species occupy opposite environmental extremes. *Cyprinodon atrorus* inhabits environmentally severe marshes and lagunas, whereas *C. bifasciatus* occurs in environmentally amenable thermal springs and their most environmentally buffered outflows. In intervening, environmentally intermediate habitats, these species hybridize extensively (i.e., dominated by advanced backcrosses). Within hybrid zones, *C. bifasciatus* is thought to expand its range downstream in spring and summer as thermal spring-like conditions extend farther downstream. In winter, *C. bifasciatus* is thought to contract its range in response to colder conditions that prevail downstream. It is unknown how hybrid genotypes, particularly *C. bifasciatus*-like hybrids, respond to seasonal environmental change; however, it is expected that *C. bifasciatus*-like hybrids will demonstrate similar patterns to *C. bifasciatus* if exogenous selection is important in this system.

To address the role of environment in hybridization between *C. atrorus* and *C. bifasciatus* this study asked three major questions. First, how important, historically, has hybridization been in *C. atrorus* and *C. bifasciatus*? Answers to this question set a foundation for a more thorough investigation of the role of environment in hybridization between these species. Second, is there evidence that these species differ in their environmental tolerances? Presence of such differences would open the possibility that hybridization is regulated at least in part by environmental factors. Third, do hybrid populations predictably respond to seasonal environmental change? Such a predictable response would demonstrate that hybridization between *C. atrorus* and *C. bifasciatus* is regulated at least in part by exogenous selection.

How Important has Hybridization been for Cyprinodon atrorus *and* C. bifasciatus?

At the nuclear gene level, we found *Cyprinodon atrorus* and *C. bifasciatus* to be well differentiated, with only minor introgression into parental populations. Introgression was only detected in populations where hybridization is known. In populations where *Cyprinodon* show considerable morphological evidence of hybridization, introgression was substantial. This pattern is in agreement with the morphological distinctiveness of these species, with localized regions of hybridization.

In contrast to nuclear genes, introgression has been substantial at the mitochondrial gene level, as all *C. bifasciatus* exhibit mtDNAs derived from *C. atrorus*. Subsequent to this replacement there has been haplotypic diversification associated with geography rather than morphology. This result restricted use of mitochondrial markers for study of hybridization between *C. atrorus* and *C. bifasciatus*, though these markers were useful for detecting what is likely ongoing secondary mitochondrial replacement in the *C. bifasciatus* from the Rio Churince. Together, these patterns demonstrate the importance of understanding hybridization in these species in order to accurately interpret genetic variation within *C. atrorus* and *C. bifasciatus*, particularly the latter.

Do these Species Differ in their Environmental Tolerances?

Cyprinodon atrorus and *C. bifasciatus* have long been considered to differ in their environmental tolerances, with *C. atrorus* thought to be euryplastic and *C. bifasciatus* stenoplastic. However, these suspected differences have never been experimentally

confirmed. Such confirmation is critical to understanding the role of environment in regulating hybridization between these species. To address the suspected environmental differences between *C. atrorus* and *C. bifasciatus*, reciprocal transplant experiments were conducted in summer 2002 and winter 2003. Results from these experiments confirm the euryplasticity of *C. atrorus* and stenoplasticity of *C. bifasciatus*. Specifically, *C. atrorus* demonstrated high survival in both its native habitat as well as the habitat of *C. bifasciatus*, whereas, *C. bifasciatus* exhibited significantly higher mortality in the severe *C. atrorus* habitat in both winter and summer that in its native habitat. These results indicate that *C. bifasciatus* has a low tolerance for environments that deviate from thermal spring conditions, whereas *C. atrorus* is physiologically well suited to severe as well as environmentally favorable conditions. These results help explain the distribution of *C. bifasciatus* (and *C. bifasciatus*-like individuals) within the hybrid zone. However, these results do not explain why *C. atrorus* is absent from *C. bifasciatus* habitat. *Cyprinodon atrorus* is likely excluded from *C. bifasciatus* habitat due to such factors as high susceptibility to predators and/or poor competitive ability. This study demonstrates the complexity of environmental factors in shaping hybrid zone dynamics between these species. However, the significance of endogenous selection in this system remains unknown.

Do Hybrid Populations Predictably Respond to Environmental Change?

If exogenous selection operates in the hybrid zone, then we expected to see an influence of seasonal environmental variation on hybridization dynamics, with 1)

downstream riverine reaches should show a decrease in *C. bifasciatus* allele frequency between early and late winter (December/January vs. March) as these populations are exposed to cold temperatures, but an increase in *C. bifasciatus* alleles between late winter and summer (March vs. August) as conditions in these reaches become more physicochemically spring-like; and 2) fishes from peripheral habitats should exhibit a decrease in *C. bifasciatus* allele frequency between early and late winter (December/January and March) as these populations are exposed to cold temperatures, and an additional decrease in *C. bifasciatus* allele frequency in summer as these habitats desiccate and in turn experience increased temperature and salinity extremes. Within the Rio Churince hybrid zone, the distribution of *C. atrorus* and *C. bifasciatus* alleles was at least partially dependent on environmental variation. Equivocal support was found for prediction 1. Here, evidence for increased *C. bifasciatus* allele frequency in downstream riverine habitats in summer was ambiguous. However, there was strong support for the predicted decrease in *C. bifasciatus* allele frequency in these habitats between early and late winter (December/January vs. March). There was very strong support for prediction 2, where there was a significant decrease in *C. bifasciatus* allele frequency in peripheral habitats between early and late winter (December/January vs. March), and an additional decrease in *C. bifasciatus* allele frequency in these habitats between late winter and summer (March and August). These patterns strongly suggest that environmental factors have a considerable influence on the distribution of genetic variation in this system on both spatial and temporal scales.

Estimates of selection coefficients across the hybrid zone indicate that selection against introgression is strong. Selection also appears to vary seasonally, as cline width changes with season. For example, in the first year of the study in the lower reaches of the system there was a regular decrease in the frequency of *Cyprinodon bifasciatus* alleles between early and late winter, followed by an increase in these alleles between late winter and summer. This pattern is consistent with previously noted (Minckley 1969) expansion of *C. bifasciatus* range in summer as downstream reaches acquire spring-like thermal profiles, but contraction of *C. bifasciatus* range in winter as these downstream reaches become cold. In the second year results were ambiguous as there was no consistent contraction in cline width across genes between early and late winter, and there was no expansion of the width of the hybrid zone between late winter and summer. Why there was no contraction in winter remains unknown, but lack of expansion of the hybrid zone in summer may have been influenced by recent human modification of the headspring, which led to increased salinities throughout the system during the summer months. In the third year there again was a contraction of cline width between early and late winter, but no expansion between late winter and summer. Again, the lack of expansion in summer may have been influenced by human activity at the headspring. Nonetheless, this evidence of a cyclical pattern does suggest that exogenous (environmental) selection is critical in regulating hybridization between *C. atrorus* and *C. bifasciatus*, as such regular variation in cline width is not predicted for hybrid zones regulated primarily by endogenous factors. However, the relative roles of endogenous and exogenous selection remain unknown.

Conclusions

Results from this study indicate that within the *Cyprinodon atrorus-C. bifasciatus* system, hybridization has had a long, complex, and evolutionarily important influence. Most striking is the contrast of complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus* with the near absence of nuclear gene introgression into either species. Although the dynamics of the mitochondrial replacement are unknown, patterns observed in modern hybrid zones between these species suggest that a combination of genetic drift and behavior, rather than natural selection, could have led to replacement. Under this scenario, a numeric bias towards *C. atrorus*, coupled with a propensity for *C. bifasciatus* males (but not females) in areas of contact to mate in habitats preferred by *C. atrorus*, could have led to replacement without natural selection *for C. atrorus* mitochondria. In contrast, distinctiveness of *C. atrorus* and *C. bifasciatus* at the nuclear gene level suggests that there is strong selection against introgression of nuclear genes. Despite the low level of nuclear gene introgression into parental populations, dynamics within the Rio Churince hybrid zone suggest that there is a large environmental component to regulation of hybridization between these species. This may in part be related to findings from reciprocal transplant experiments. Here introgression of *C. bifasciatus* nuclear genes into *C. atrorus* populations may be limited due to physiological constraints that make *C. bifasciatus* poorly adapted to severe environments. Lack of *C. atrorus* nuclear genes into *C. bifasciatus* populations, in turn, is possibly related to this species poor competitive abilities and high susceptibility to predation. The

role of endogenous selection, while not quantified, may also be important in determining patterns of introgression in this system.

Reproductive isolation between *C. atrorus* and *C. bifasciatus*, while not complete, is advanced, and factors that influence this isolation appear to be complex. For example there is considerable evidence that these species are separated by environmental tolerance differences, which likely affect the fitness of hybrid offspring. In addition, behavioral differences such as predation resistance and competitive ability appear vastly different between these species, with *C. bifasciatus* being superior in both respects. It is also likely that intrinsic selection against hybrids is critical to the maintenance of reproductive isolation, as evidenced by coincidence and concordance of nuclear gene clines across, and significant linkage disequilibrium within, the studied hybrid zone. Thus, although the relative importance of endogenous and exogenous selection remain unknown, it is probable that both types of selection are important in maintaining differentiation between these species.

Future research should focus on several major areas. First, extended analysis of the role of physicochemical variation on determining hybrid zone structure should be addressed. In particular, physiological experiments should be conducted to identify the mechanistic bases of environmental tolerance differences between *C. atrorus*, *C. bifasciatus*, and various classes of hybrids. This will allow more precise establishment of the mechanisms that limit introgression of *C. bifasciatus* alleles into *C. atrorus* populations. Focus also needs to be placed on factors that prevent *C. atrorus* from inhabiting *C. bifasciatus* habitats. To address this phenomenon, experiments should be

conducted to quantify *C. atrorus* resistance to predation, relative to that of *C. bifasciatus*. Competitive differences between *C. atrorus* and *C. bifasciatus* also need to be addressed, as these place important limitations on the distribution of *C. atrorus*. Once these differences in predation resistance and competitive ability are addressed with *C. atrorus* and *C. bifasciatus*, similar studies should be expanded to include various classes of hybrids. Combination of these studies will add to the understanding of basic ecological and fitness differences between parental species, and between hybrids and parental species. In turn, these studies will allow for determination of whether some classes of hybrids exhibit increased fitness relative to parental species. Finally, the role of endogenous selection in this system needs to be quantified to determine relative importance of exogenous and endogenous selection. Together, these studies will build upon existing research to provide a robust understanding of the dynamics and evolutionary importance of hybridization between *C. atrorus* and *C. bifasciatus*.

Findings from this study have important implications for understanding the role hybridization may play in evolution. This role is most clear at the mitochondrial level, where hybridization has led to the complete replacement of *C. bifasciatus* mitochondrial genome by that of *C. atrorus*. Relative to mitochondrial variation, this study has also demonstrated the critical role genetic drift may play in evolution. While nuclear gene introgression was limited outside present day hybrid zones, association between nuclear gene variation and environmental variables indicates that within hybrid zones there is substantial importance of environmental change on the regulation of hybridization between *C. atrorus* and *C. bifasciatus*. Predictable association between nuclear gene

variation and seasonal environmental change indicates that local selection within the hybrid zone may occur. This not only suggests that environmental variation is important to regulation of hybridization between *C. atrorus* and *C. bifasciatus*, but also indicates that environmental variation may play a critical role in governance of hybridization in other animal systems. This study also demonstrated the importance of assessing both spatial and temporal influences on hybrid zone dynamics. Future studies of hybrid zones should account for temporal, as well as spatial, patterns that may influence hybrid zone structure and dynamics.

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