Phylogeography of the longnose dace (*Rhinichthys cataractae*) species group in northwestern North America — the origin and evolution of the Umpqua and Millicoma dace

J.D. McPhail and E.B. Taylor

Abstract: The Umpqua and Millicoma dace are small cyprinid fishes endemic, respectively, to the Umpqua and Coos rivers on the central coast of Oregon. The origins and relationships of these dace are unclear; however, two hypotheses have been postulated that assume these dace had evolved from a longnose dace (*Rhinichthys cataractae* (Valenciennes, 1842)) like ancestor, but from different modes of origin. The direct origin hypothesis postulates that each of these dace originated directly, but independently, from a common ancestor. In contrast, the indirect origin hypothesis postulates that the Umpqua dace originated from a *R. cataractae* like ancestor and that the Millicoma dace evolved from the Umpqua dace. We used mitochondrial (cytochrome *b* and control region) sequences to test the two hypotheses. Our maximum likelihood analysis supports the indirect origin hypothesis and argues that together the Umpqua and Millicoma dace form a distinctive Oregon coastal clade within the *R. cataractae* species group. We also attempt to reconcile this result with the observation that the geographic distribution of the morphologically divergent Umpqua dace is sandwiched between the geographic ranges of the morphologically similar Millicoma dace and longnose dace.

Résumé : Les naseux de l'Umpqua et de la Millicoma sont de petits poissons cyprinidés endémiques, respectivement dans les rivières Umpqua et Coos de la côte centrale de l'Oregon. L'origine et les relations de ces naseux sont incertaines; on a cependant élaboré deux hypothèses qui présupposent que ces naseux ont évolué à partir d'un ancêtre de type naseux des rapides (*Rhinichthys cataractae* (Valenciennes, 1842)), mais par des modes d'origine différents. L'hypothèse d'origine directe énonce que chacun de ces naseux tire son origine directement, mais indépendamment, d'un ancêtre commun. En revanche, l'hypothèse d'origine indirecte énonce que le naseux de l'Umpqua provient directement d'un ancêtre de type *R. cataractae* et que le naseux de la Millicoma a évolué à partir du naseux de l'Umpqua. Nous avons testé les deux hypothèses à l'aide de séquences mitochondriales (cytochrome *b* et région de contrôle). Une analyse de vraisemblance maximale appuie l'hypothèse d'origine indirecte et indique qu'ensemble les naseux de l'Umpqua et de la Millicoma forment un clade distinct de la côte d'Oregon au sein du groupe d'espèces de *R. cataractae*. Nous essayons aussi de réconcilier ce résultat avec l'observation que la répartition géographique des naseux de l'Umpqua, qui sont différents par leur morphologie, se trouve insérée entre les répartitions géographiques des naseux de la Millicoma et des naseux des rapides qui se ressemblent morphologiquement.

[Traduit par la Rédaction]

Introduction

The longnose dace (*Rhinichthys cataractae* (Valenciennes, 1842)) has the widest geographic distribution of any native North American minnow (Cyprinidae). Longitudinally, it occurs in inland waters from the Atlantic to the Pacific coasts and, latitudinally, from north of the Arctic Circle south to northern Mexico. Given this vast geographic range and the concomitant opportunities for allopatric divergence, it is surprising that only one allopatric species — the Umpqua dace (*Rhinichthys evermanni* Snyder, 1908) — appears to be derived from the longnose dace.

The Umpqua dace's geographic range is restricted: it occurs in two small drainage systems (the Umpqua and Smith rivers) that share a common estuary on the central Oregon coast (Snyder 1908). In the same area, however, there is another *R. cataractae* like dace, the Millicoma dace. It is restricted to the Coos River, a small drainage system adjacent to, and immediately south of, the Umpqua River (Fig. 1). The origin and relationships of the Millicoma dace to longnose and Umpqua dace are unclear.

Bisson and Reimers (1977) assumed that both the Umpqua and Millicoma dace evolved from an *R. cataractae* like ancestor derived from the Columbia River system. This assumption is based on two observations: (1) the Umpqua River was once a Columbia River tributary (Baldwin 1981) and (2) the Columbia River is the only geographically adjacent source of an *R. cataractae* like ancestor. Bisson and Reimers (1977) compared the morphology of the Millicoma dace with populations of *R. cataractae* from the Columbia

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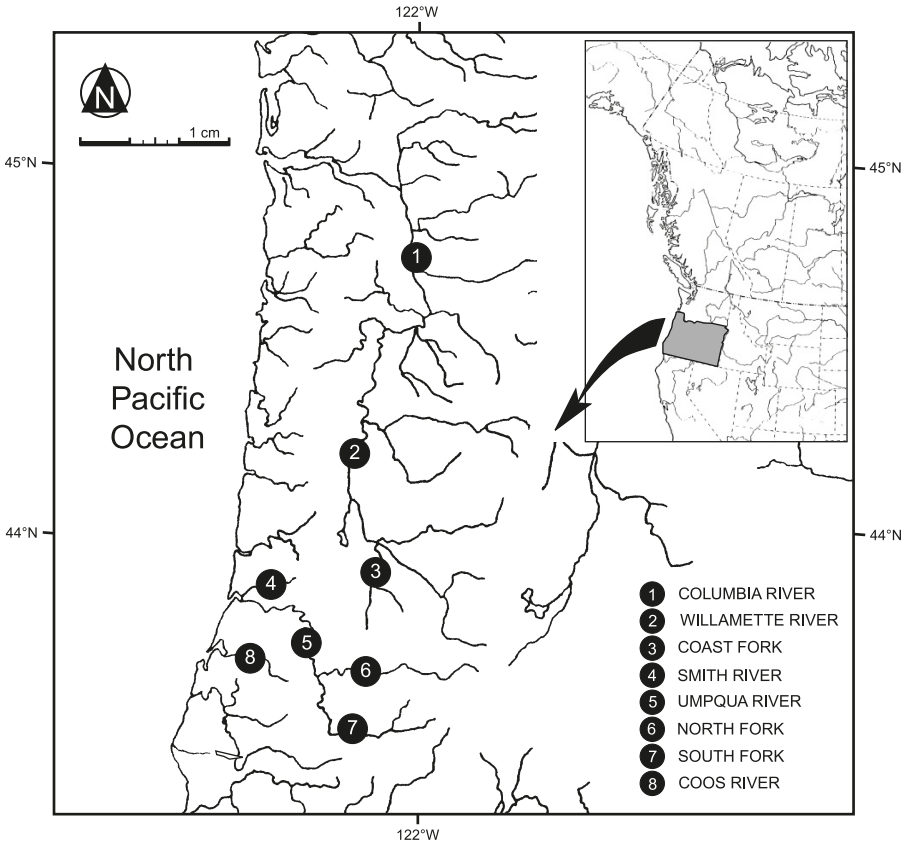
Fig. 1. Map of the central Oregon coast showing the rivers mentioned in our study. The shaded area in the inset indicates the position of Oregon on the west coast of North America. Scale: 1 cm = 36.8 km.



River system and with *R. evermanni* from the Umpqua River. Their analysis indicated that the Umpqua dace differed from typical northwestern longnose dace in body proportions, scale counts, and in the number of dorsal fin rays. In contrast, the Millicoma dace closely resembled *R. cataractae* in body proportions, scale counts, and in the number of dorsal fin rays. The morphological distinctness of the Umpqua dace and the morphological similarity of longnose and Millicoma dace poses a biogeographic and phyletic problem. How did the morphologically divergent Umpqua dace come to be geographically sandwiched between the morphologically similar, but not identical, longnose and Millicoma dace?

Bisson and Reimers (1977) suggested two hypotheses to explain the origin and distributions of Umpqua and Millicoma dace. One hypothesis postulates that the Millicoma dace evolved directly from an *R. cataractae* like ancestor (i.e., its origin was independent of the origin of the Umpqua dace). Their alternative hypothesis proposes an initial colonization of the Umpqua River by an *R. cataractae* like ancestor, followed by a period of isolation and divergence in the Umpqua River and a later dispersal into the Coos River. Under this hypothesis the origin of the Millicoma dace is not independent of the origin of the Umpqua dace but, instead, evolved from an early form of the Umpqua dace.

These hypotheses make different predictions about the mitochondrial relationships among northwestern longnose (*R. cataractae*), Umpqua (*R. evermanni*), and Millicoma dace. The independent origin hypothesis postulates the dispersal of an *R. cataractae* like ancestor from the Columbia River system directly into the Coos drainage system. Under this hypothesis, the expected mitochondrial relationships of Millicoma, Umpqua, and longnose dace depend on the timing of this hypothetical dispersal event. Since this hypothesis postulates that Millicoma and Umpqua dace were derived independently from an *R. cataractae* like ancestor, both taxa should be phyletically related to *R. cataractae*; however, they should not be sister taxa.

The alternative (dependent origin) hypothesis postulates that an *R. cataractae* like progenitor initially colonized the Umpqua River, where presumably some differentiation occurred, and then later dispersed into the Coos River system. Under this scenario, the expected mitochondrial relationships of Millicoma, Umpqua, and longnose dace depend on when the ancestral Umpqua dace reached the Coos River system. If it reached the Coos River relatively recently (i.e., late in the Pleistocene), Millicoma and Umpqua dace should share most of the mitochondrial sites that differentiate the Umpqua dace from modern Columbia longnose dace, and the depth of the sequence divergence between Millicoma and Umpqua dace should be relatively shallow (not much more than 0.5%). If, however, the colonization of the Coos River from the Umpqua system occurred earlier (in the late Pliocene or early Pleistocene), Umpqua and Millicoma dace should still share some sites that differentiate them from modern Columbia longnose dace and the depth of the sequence divergence between Millicoma and Umpqua dace should be >1.0%.

In the present study, we use mitochondrial sequence data from Columbia system longnose dace, Umpqua dace, and Millicoma dace to examine these hypotheses and make inferences about the origins and relationships of Umpqua and Millicoma dace.

Materials and methods

Sampling

The dace used in this study were anesthetized with tricaine methanesulfonate before preservation in 95% ethanol. Voucher specimens have been deposited in the University of British Columbia Fish Museum. The cytochrome *b* gene (1140 base pairs) and the control region (1008 base pairs) were sequenced in longnose, Umpqua, and Millicoma dace (a total of 60 fish; Table 1). Five longnose dace (*R. cataractae*) were sequenced from each of five Columbia River sites — two lower Columbia sites on the Coast Fork of the Willamette River, two middle Columbia sites (Shitike Creek, Deschutes River, Oregon, and Otter Creek, Similkameen River, British Columbia), and one upper Columbia site (the river's source: Columbia Lake, British Columbia). Five Umpqua dace (*R. evermanni*) were sequenced from each of three sites in the Umpqua River system — one site each on the North and South forks of the Umpqua River, and one site on the Smith River. In addition, five Millicoma dace were sequenced from a single site on the Coos River — the Millicoma rest area.

To provide an outgroup for our phylogenetic analysis, we also sequenced three northwestern species in the speckled dace species group: five speckled dace (*Rhinichthys osculus* (Girard, 1856)) from the Kettle River at Midway, British Columbia; five leopard dace (*Rhinichthys falcatus* (Eigenmann and Eigenmann, 1893)) from the Fraser River near Agassiz, British Columbia, and five Umatilla dace (*Rhinichthys umatilla* (Gilbert and Evermann, 1894)) from a site at the confluence of the Slocan and Kootenay rivers near Castlegar, British Columbia.

Morphology

Our specimen collections were small (usually five individuals per site) and consisted of alcohol-preserved specimens. Thus, the sample sizes are too small and too distorted to provide a meaningful analysis of either body proportions or shape. Consequently, the morphological comments in our study are based (Table 1) on Bisson and Reimers (1977).

DNA extraction

Following the manufacturer's protocols, DNA was extracted using a Puregene™ DNA isolation kit. The samples consisted of ethanol-fixed fin or liver tissue.

Primers and amplification

The primers used to amplify both cytochrome *b* and the control region were modifications of the "universal" primers described in Kocher et al. (1989). In both cases, the size of the gene required two overlapping sequences to obtain the complete gene. The 5′ half of the cytochrome *b* gene was amplified and sequenced using the following primers: 5′-TGA CTT GAA GAA CCA CCG TTG-3′ and 5′-AGG GGT GAG AGT TAA AAT CTC-3′, while the 3′ half of the gene was sequenced with 5′-TGA GGA CAA ATA TCC TTT TGA GGC-3′ and 5′-AGG GGT GAG AGT TAA AAT CTC-3′. Amplifications were carried out in 50 μL of total volumes containing (final concentrations) 200 mmol/L each of dATP, dGTP, dCTP, and dTTP; 800 nmol/L of each primer; 6 U (1 U ≈ 16.67 nkat) of *Taq* polymerase; 6.7 mmol/L of Tris-HCl (pH 8.8); 1.0 mmol/L of 2-mercaptoethanol; 2.2 mmol/L of $MgCl_2$; and between 10 (purified mtDNA) and 1000 ng (genomic DNA) of template. Thirty-five amplification cycles were performed under the following conditions: denaturation at 95 °C for 60 s, primer annealing at 48 °C for 60 s, and primer extension at 72 °C for 90 s. A final single extension step was performed at 72° for 5 min.

The control region also was amplified and sequenced using two overlapping sets of primers: 5′-GAG ATT TTA ACT CTC ACC CCT-3′ and 5′-TGA GGA GTA TGT AAT TAC ACC-3′, and 5′-CAT GAT AGA ATC AGG GAC ACA-3′ and 5′-GTG GCT GGG ACG AGT TTT ACC GGC-3′. A variable number of tandem repeats (VNTR) near the 3′ end of the control region forced us to sequence this region from both the 5′ and 3′ ends. The amplification procedure was the same as for the cytochrome *b* gene except for the annealing temperatures: 52 °C for the 5′ region and 56 °C for the 3′ region, respectively.

After the sequencing PCR, dye terminators were removed from the double-stranded product using Centri-sep™ columns and the product was sequenced on an ABI model 377 automatic sequencing machine (Applied Biosystems (ABI), Foster City, California).

Sequences and analyses

Complete cytochrome *b* and control region sequences for longnose (Coast Fork of the Willamette River), Umpqua, and Millicoma dace are deposited in GenBank (accession nos.): FJ744108, EU780890, EU871709 (cytochrome *b*) and FJ69178, EU791457, EU797189 (control region). The accession numbers for the cytochrome *b* sequences of Umatilla, leopard, and speckled dace are FJ48865, FJ69176, and FJ69177, respectively. The accession numbers for the control region of the of Umatilla, leopard, and speckled dace are FJ69179, FJ748865, and FJ748866, respectively. The only variation detected within our Umpqua and Millicoma dace sequences were occasional G–A or C–T transitions; however, in longnose dace, there is considerable geographically patterned mitochondrial variation within the Columbia River system. Our sample sizes (and their geographic distribution) are too small to speculate on this variation. Typically, however, within small rivers, this variation is minor and consists of occasional transitions and the interpopulation genetic distances are small (<0.5%). In contrast, interspecific sequences typically contain transversions, as well as transitions, and interspecific genetic distances were typically >1.5%. Although deletions and insertions usually were consistent within species, there are two VNTRs (variable number of tandem repeats) in the *Rhinichthys* control region. One VNTR is composed of GT repeats. The pattern and number of repeats in this VNTR consistently differs between the longnose and speckled dace species groups — six repeats in the longnose dace species group and three in the speckled dace species group. The other VNTR is made up

Table 1. List of drainage systems, names of sampling sites, species abbreviations, and coordinates (latitude and longitude) used in the tables and figures of our study.

River system	Site name	Coordinates
Rhinichthys cataractae		
Lower Columbia (LCOL)	Coast Fork, Willamette River	43°36′55″N, 123°04′52″W
	Row River, Willamette River	43°47′24″N, 123°01′25″N
Middle Columbia (MCOL)	Shitike Creek, Deschutes River	44°52′14″N, 121°01′29″W
	Otter Creek, Similkameen River	49°38′19″N, 120°46′58″W
Upper Columbia (UCOL)	Columbia Lake, Columbia River	52°06′08″N, 115°49′08″W
Rhinichthys evermanni **(REVE)**		
Umpqua River (REVEN)	Whistlers Bend, north fork	43°18′39″N, 123°13′04″W
Umpqua River (REVES)	Winston, south fork	43°07′12″N, 123°26′03″W
Umpqua River (RSMIT)	Smith River, Smith Falls	43°47′24″N, 123°48′47″W
Millicoma dace (RMILL)		
Coos River	Millicoma rest area	43°26′38″N, 123°59′35″W
Rhinichthys osculus **(ROSC)**		
Middle Columbia	Kettle River at Midway	49°00′19″N, 118°46′30″W
Rhinichthys falcatus **(RFALC)**		
Lower Fraser	Big Island Bar near Agassiz	49°12′14″N, 121°47′08″W
Rhinichthys umatilla **(RUMAT)**		
Upper Columbia	Kootenay River confluence with Columbia River	49°20′10″N, 117°49′ 02″W

of TA repeats. Again, the number of repeats differs between the longnose and speckled dace species groups: 11–14 repeats in the longnose dace species group and 8–9 repeats in the speckled dace species group. Within the Columbia River system and associated drainages (i.e., drainages that contain a fish fauna derived from the Columbia system), the number of TA repeats varies within and among allopatric populations of longnose dace. Thus, within this group, the TA repeats may be a useful phylogeographic marker; however, a detailed analysis will require larger sample sizes from a wider geographic area than are currently now available. Regardless of their length, in the present analysis we treat all insertions or deletions in this VNTR as single characters.

All analyses of sequence data were performed using algorithms in PAUP version 4.0b8w (Swofford 1998). Sequences were aligned using the CLUSTAL algorithm (Higgins et al. 1996) and pairwise genetic distances were calculated from the sequence data. Appropriate substitution models were obtained using MODELTEST version 3.03 (Posada and Crandall 1998). Two substitution models were used in our analyses: the Tamura–Nei model for the cytochrome *b* data (Tamura and Nei 1993) and the HKY85 model (Hasegawa et al. 1985) for the control region. Both models were corrected for back mutations. In addition to calculating genetic distances, we also performed parsimony and maximum likelihood analyses on our sequences. The topologies of the trees obtained from these analyses were identical. Consequently, only the maximum likelihood phylograms (Figs. 2A, 2B) are presented. To assess confidence in the nodes, the data matrices were bootstrapped ($N = 1000$) using the heuristic search option in PAUP.

Estimating divergence times

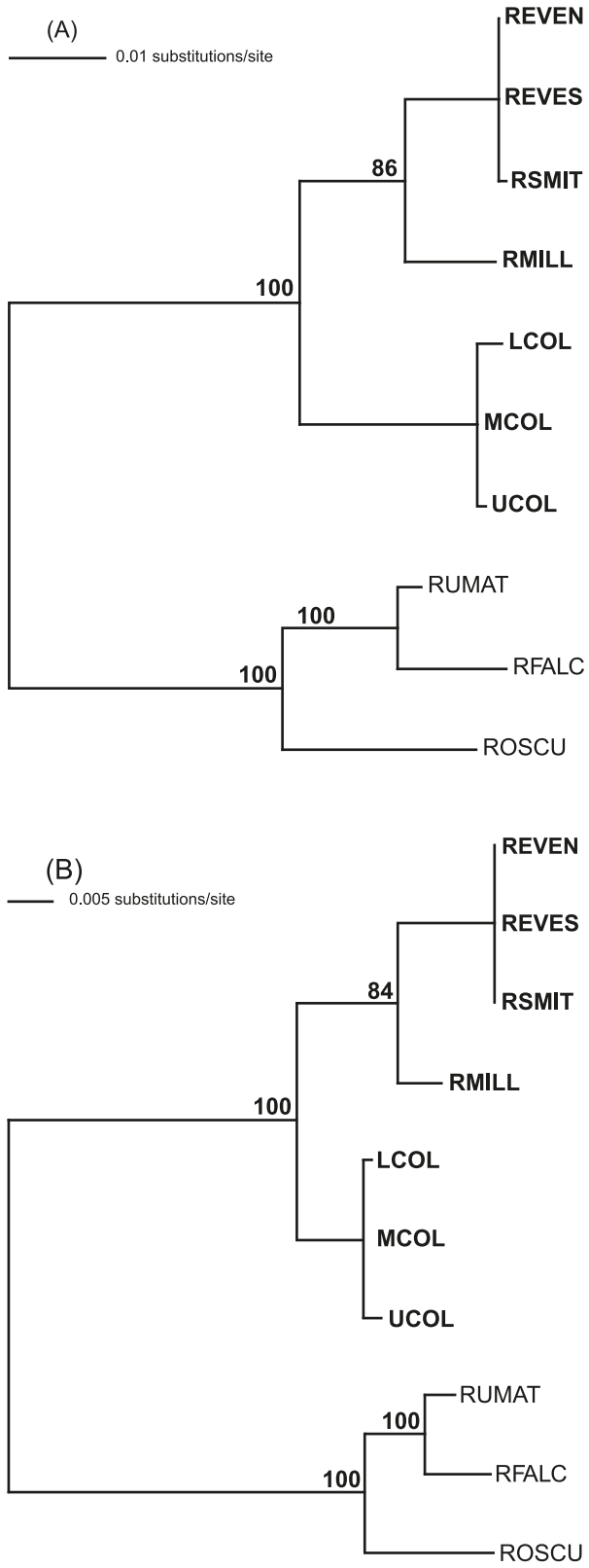
Estimating divergence times from sequence data can be complex (Arbogast et al. 2002); however, by matching fossil data with sequence data (cytochrome *b*), Smith et al. (2002) were able to estimate divergence times in several groups of Great Basin fishes. Their analysis indicates divergence rates vary among groups (e.g., 0.5% per Ma (million years) in salmonids versus 1.0% per Ma in minnows (Cyprinidae)). We used the cyprinid rates in Smith et al. (2002) to estimate divergence times for our data.

Results

Genetic distances calculated from cytochrome *b* and control region sequences are presented in Table 2. These data gave a broad range of genetic distances: from 0.0% to 0.6% among conspecific populations to >12% among species groups. Bootstrap resampling of our sequences provides strong support (86%–100%) for the presence of three divergent groups within our data (Figs. 2A, 2B). Not surprisingly, the most divergent of these groups (with a mean divergence of 10.3%) is our outgroup: the speckled dace species group. There are three species in our outgroup — the speckled dace (*R. osculus*), the leopard dace (*R. falcatus*), and the Umatilla dace (*R. umatilla*). Within this group the speckled dace is the most divergent species (2.5%–4.5%), while the leopard and Umatilla dace (1.1%–1.4%) are more closely related to each other than to the speckled dace.

Within our ingroup (the *R. cataractae* species group), there is strong (>84%) bootstrap support for two subgroups — a northwestern longnose clade and an Oregon coastal clade (Figs. 2A, 2B). Within our Columbia Basin samples, the genetic distances among lower, middle, and upper Columbia populations are relatively small (<0.6%) and the differences consist entirely of transitions. In contrast, the divergence between Columbia Basin longnose dace and Oregon coastal *R. cataractae* like dace averages about 3.4% (Table 2), and is made up of both transitions and transversions. Within the Oregon coastal subgroup there is a well-supported (100%) separation into two forms

Fig. 2. Maximum likelihood phylograms for cytochrome *b* (A) and the control region (B). The longnose dace species group is in boldface type. The speckled dace species group is the outgroup. The numbers at the nodes are the bootstrap values (N = 1000). Species codes are given in Table 1.



(Figs. 2A, 2B): the Umpqua dace and the Millicoma dace. Again, interpopulation divergences among the three Umpqua sampling sites (including the Smith River site) are small (<0.2%), whereas the Millicoma dace differs from the Umpqua dace by 1.7%–2.0%.

Discussion

Geological setting

The Columbia River is the largest and oldest drainage system in northwestern North America. It has existed in roughly its present course for about 12 Ma (McKee 1972). Consequently, the Columbia River predates the uplift of both the Cascade and Coast mountain ranges. In contrast, most of the independent drainage systems along the Oregon coast rise on the western slope of the Coast Mountains (Fig. 1), and this implies that these rivers postdate the late Pliocene or early Pleistocene uplift of the Coast Mountains (McKee 1972). An exception to this generalization is the largest of the Oregon central coast rivers: the Umpqua River. It rises on the western slope of the Cascade Range and flows through the Coast Mountains (Fig. 1). Thus, although the Umpqua River was once tributary to the Columbia River (by way of the ancestral Willamette River), it existed as an independent drainage system before the uplift of the Coast Mountains. Baldwin (1981) indicates that the modern Umpqua drainage system separated from the Columbia River system sometime in the late Cenozoic, and Priest et al. (1983) date the establishment of the major drainage patterns on the western slope of the Cascade Mountains at 3–4 Ma. In contrast to the Umpqua River, the Coos River drains the western slope of the Coast Mountains, and this argues that the Coos drainage system originated sometime (late Pliocene or early Pleistocene) after the rise of the Coast Mountains.

Origin and evolution of the Millicoma dace

We attempted to test the two hypotheses suggested by Bisson and Reimers (1977) to explain the origin of the Umpqua and Millicoma dace. The Bisson and Reimers independent origins hypothesis argues that both these dace originated directly from an *R. cataractae* like ancestor. Consequently, both should be phyletically related to Columbia longnose dace but should not be sister taxa. Additionally, since the connection between the Umpqua and Columbia drainage systems was severed in the mid-Pliocene and the Coos drainage system did not form until the late Pliocene or early Pleistocene uplift of the Coast Mountains, Umpqua and Millicoma dace should also differ in the depth of their mitochondrial divergences from Columbia longnose dace.

Bisson and Reimers' (1977) alternative (dependent origin) hypothesis postulates that an *R. cataractae* like progenitor was isolated in the Umpqua River when the Umpqua River became an independent drainage system sometime in the mid-Pliocene. Much later, in the late Pliocene or early Pleistocene, the progenitor of the present Umpqua dace dispersed into the newly formed Coos drainage system. This hypothesis predicts that the Umpqua and Millicoma dace should be sister taxa; however, the depth of divergence between the two taxa would depend on when the ancestral Umpqua dace

Table 2. Pairwise genetic distances among northwestern members of the *Rhinichthys cataractae* (in boldface type) and *Rhinichthys osculus* (in regular type) species groups.

	REVEN	REVES	RSMIT	RMILL	LCOL	MCOL	UCOL	RUMAT	RFALC	ROSCU
REVEN	—	**0.000**	**0.000**	**0.017**	**0.031**	**0.032**	**0.032**	0.119	0.129	0.134
REVES	**0.000**	—	**0.000**	**0.017**	**0.031**	**0.032**	**0.032**	0.119	0.129	0.134
RSMIT	**0.001**	**0.001**	—	**0.017**	**0.031**	**0.032**	**0.032**	0.119	0.129	0.134
RMILL	**0.019**	**0.019**	**0.020**	—	**0.024**	**0.025**	**0.025**	0.115	0.122	0.127
LCOL	**0.039**	**0.039**	**0.040**	**0.041**	—	**0.001**	**0.003**	0.102	0.108	0.114
MCOL	**0.037**	**0.037**	**0.038**	**0.038**	**0.002**	—	**0.002**	0.102	0.108	0.116
UCOL	**0.038**	**0.038**	**0.038**	**0.039**	**0.003**	**0.001**	—	0.098	0.105	0.113
RUMAT	0.086	0.086	0.087	0.076	0.088	0.086	0.087	—	0.011	0.025
RFALC	0.093	0.093	0.094	0.083	0.092	0.091	0.092	0.014	—	0.029
ROSCU	0.093	0.093	0.094	0.085	0.090	0.088	0.088	0.034	0.043	—

Note: Cytochrome *b* values are below the diagonal and the control region are above the diagonal. Species codes are given in Table 1.

reached the Coos River. If it reached the Coos River relatively recently (i.e., late in the Pleistocene), Millicoma and Umpqua dace should share most of the mitochondrial sites that differentiate the Umpqua dace from modern Columbia longnose dace, and the depth of the divergence between Millicoma and Umpqua dace should be relatively shallow (not much more than 0.5%). If, however, the colonization of the Coos River from the Umpqua system occurred earlier (in the late Pliocene or early Pleistocene), Umpqua and Millicoma dace should still share some sites that differentiate them from modern Columbia longnose dace, but the depth of the divergence between Millicoma and Umpqua dace should be >1.0%.

Our genetic distance data (Table 2) do not support the independent origin hypothesis. They are, however, compatible with the dependent origin hypothesis. Umpqua and Millicoma dace share enough sites to unite them into a distinctive Oregon coastal clade within the *R. cataractae* species group (Figs. 2A, 2B). This argues that Umpqua and Millicoma dace are sister taxa. Also, the genetic distances separating this Oregon coastal clade from Columbia longnose dace (2.4%–4.1%; Table 2) are consistent with the geological evidence for the separation of the Columbia and Umpqua drainage systems about 3–4 Ma (Priest et al. 1983). Within the Oregon coastal clade, the divergence between Millicoma and Umpqua dace ranges from 1.7% to 2.0% (Table 2). This level of divergence is about 10 times greater than the divergences typically found among allopatric populations of both Umpqua and northwestern longnose dace (Table 2) and suggests that dispersal of ancestral Umpqua dace into the Coos River occurred about 1.5–2.0 Ma.

Interestingly, the morphology of Millicoma dace, especially body shape, fin ray number, and scale counts, resembles longnose dace more closely than they resemble Umpqua dace (Table 1 in Bisson and Reimers 1977). Given that Umpqua and Millicoma dace are sister taxa, and that the Millicoma dace evolved from a progenitor of the modern Umpqua dace, the morphological similarity of modern Millicoma and modern northwestern longnose dace is curious. Perhaps the distinctive morphology of modern Umpqua dace did not evolve until after the Coos River system was colonized and gene flow between the two systems was severed. Thus, isolated in the relatively small and depauperate Coos drainage system, the Millicoma dace may have retained some of the ancestral characteristics of the progenitor of both the modern Umpqua and Millicoma dace. In contrast, the Umpqua dace probably continued to evolve in the much larger and biologically more complex Umpqua drainge system. Although this scenario is speculative, the alternative explanation — the re-evolution of a *R. cataractae* like morphology from an ancestor that was morphologically similar to the modern Umpqua dace — is less parsimonious.

Taxonomic status of the Millicoma dace

Our data argue that the Umpqua and Millicoma dace are sister taxa that together they form a distinctive Oregon coastal clade within the longnose dace species group. This raises the question of the taxonomic status of the Millicoma dace. Clearly, the Millicoma dace has diverged from the Umpqua dace but is this divergence sufficient to warrant specific status? For allopatric taxa, decisions on specific status often are matters of judgment. In this case, we think that the Millicoma dace warrants specific status. Two lines of evidence support this opinion. First, although the cytochrome *b* and the control region sequence divergences between Millicoma and Umpqua dace are not deep (1.7%–2.0%), they are deeper than the same divergences (1.1%–1.4%) between other taxonomically recognized western species of *Rhinichthys* (e.g., the leopard dace (*R. falcatus*) and the Umatilla dace (*R. umatilla*); Table 2). Second, there is at least one morphological trait (dorsal fin ray number) that distinguishes all Umpqua dace from all northwestern longnose dace and all Millicoma dace — 9 dorsal rays in the Umpqua dace (N = 35) and 7–8 in both northwestern longnose (N = 48) and Millicoma dace (N = 77).

In summary, our data indicate that Millicoma and Umpqua dace originated from an *R. cataractae* like ancestor. The initial separation from the longnose dace lineage probably occurred when the Umpqua River became a drainage system separate from the Columbia drainage (sometime in the early to mid-Pliocene). With the uplift of the Coast Mountains (in the late Pliocene or early Pleistocene), the Coos drainage system formed and was colonized by the progenitor of the modern Umpqua dace. Presumably, the connection between the Umpqua and Coos drainage systems was short-lived and thereafter the two dace diverged into allopatric sister species.

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