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A B S T R A C T

The *Galaxias vulgaris* species complex comprises a morphologically and genetically diverse complex of lineages, distributed in the eastern South Island, New Zealand. It ranges from the upper reaches of the Waiau and Motueka Rivers in the north to streams of Southland and also in Stewart Island streams, in the far south. Multivariate analysis of morphological and meristic data from 396 fish of this species group from 44 populations in the eastern South Island failed to group the populations in a way consistent with grouping using molecular data. This suggests that there will be serious problems in both clarifying the taxonomy of these populations and in identifying fish from these populations in the field.

Keywords: *Galaxias vulgaris*, freshwater fish, morphotypes, genetic lineages, Canterbury, Otago, New Zealand

1. Introduction

The *Galaxias vulgaris* species complex (Allibone et al. 1996) comprises a morphologically and genetically diverse complex of lineages, distributed in the eastern South Island from the upper reaches of the Waiau and Motueka Rivers in the north to streams of Southland and also in Stewart Island streams, in the far south. Populations are known west of the Southern Alps only in tributaries of the Maruia River, an inland part of the west-flowing Buller River system.

Recent taxonomic and genetic studies of these lineages have resulted in the description or redescription of several additional species, with an increase in the number of species formally recognised from one (McDowall 1970, 1990) to five (McDowall & Wallis 1996; McDowall 1997; McDowall & Chadderton 1999). Molecular studies have revealed several additional genetic lineages, some of which may warrant recognition as distinct species (Waters & Wallis 2000, 2001a, 2001b; Waters et al. 1999, 2001a, 2001b; Esa et al. 2001; Wallis et al. 2001). The lineage complexity of these populations is particularly evident in the river systems of Otago and Southland. Taxonomic decisions are yet to be made about which, if any, further lineages should be regarded as distinct species.

Regardless of whether and which lineages are finally elevated to species status, there are serious problems in allocating specimens or populations discovered to the various lineages and species, either in the field or the laboratory. These problems are generated by a combination of variation within and among lineages and close similarities between them.

The present projects are aimed at obtaining morphological data from populations/lineages/described species, and subjecting these data to multivariate statistical analyses to determine whether there are morphological characters that are of practical use in assigning populations/individuals to the various recognised species or lineages.

Morphological data used for this purpose comprised either:

- Those collected for published studies on the systematics of this species complex (McDowall & Wallis 1996; McDowall 1997; McDowall & Chadderton 1999),

or:

- Additional data specifically obtained for the present studies.

Additional data were derived from samples collected from populations carefully chosen to provide representation of the various taxa and lineages identified from molecular studies and after discussion with Richard Allibone of DOC (and relevant to the genetic studies of Wallis, Waters and colleagues, listed below).

2. Study methods

Data were obtained from fish specimens as follows: 28 standard body dimensions were measured and counts taken of seven serially-repeated body parts (such as fin rays in the various fins – listed in Table 1). In general, ten specimens were examined from any locality, though sometimes there were fewer specimens available in samples that were suitable for study. Occasionally, more specimens were studied (Table 2), this being due to the data being derived originally for published taxonomic studies (McDowall & Wallis 1996; McDowall 1997; McDowall & Chadderton 1999).

Measurements were made with digital display callipers that provide for readings of measurements to 0.1 mm; however, it should not be assumed that this level of accuracy applies, as dimension size and accuracy of measurement are affected by several uncontrollable variables:

1. Distances measured may depend on the treatment of the fish during preservation, so that there is differential shrinkage between samples.
2. Fish may be bent when preserved, and the way they are straightened during measurement can affect some dimensions by substantially more than 0.1 mm.
3. Just the process of fixation in a specimen bottle can lead to flattening of some soft dimensions, like snout length resulting in influences on distances of more than 0.1 mm.
4. Some of the dimensions measured do not have strongly defined limits, so that their determination involves best estimates of those limits.

TABLE 1. LIST OF MORPHOMETRIC AND MERISTIC DATA ASSEMBLED FOR ANALYSIS (LARGELY AS DEFINED IN McDOWALL 1970; McDOWALL & WALLIS 1996).

Measurements	
Total length	Pectoral-pelvic length
Standard length	Pelvic-anal length
Body depth at vent	Head length
Length of caudal peduncle	Head depth
Depth of caudal peduncle	Head width
Predorsal length	Snout length
Preanal length	Postorbital head length
Length of dorsal fin base	Interorbital width
Maximum length of dorsal fin	Eye diameter
Length of anal fin base	Length of upper jaw
Maximum length of anal fin	Length of lower jaw
Pectoral fin length	Width of gape
Pelvic fin length	Depth of gape
Prepelvic length	Length of pyloric caecum

Counts	
Dorsal fin rays	Pelvic fin rays
Anal fin rays	Gill rakers on first arch
Caudal fin rays	Pyloric caeca
Pectoral fin rays	

TABLE 2. SAMPLE LOCALITIES, LINEAGES, AND SAMPLE SIZES (SPECIES). NAMES IN BOLD ARE TYPE LOCALITIES.

SAMPLE NO.	COLLECTION SITE	RIVER SYSTEM	MORPHOLOGICAL GROUP	SPECIES (IF KNOWN)	NO. OF SPECIMENS STUDIED
1	Motueka River tributary	MOTUEKA	Canterbury galaxias	?"northern"	3
2	Serpentine Creek	CLARENCE	Canterbury galaxias	Northern	8
3	Conway	CONWAY	Canterbury galaxies	Northern	10
4	First Creek, Maruia River	BULLER	Canterbury galaxias	?"northern"	10
5	**Rubicon River**	WAIMAKARIRI	Canterbury galaxias	*G. vulgaris*	10
6	Maerewhenua River	WAITAKI	Canterbury galaxias	*G. vulgaris*	10
7	Kakanui River	KAKANUI	?	?	10
8	Shag River - Pigroot	SHAG	?	?	10
9	McCormick Creek	SHAG	?	?	10
10	Healy Creek, upper	TAIERI	Roundhead	*G. anomalus*	10
11	German Creek,	TAIERI	Roundhead	*G. anomalus*	10
12	**Tributary A**, Kyeburn River	TAIERI	Flathead	*G. depressiceps*	20
13	Nenthorn/Deighton	TAIERI	Flathead	*G. depressiceps*	10
14	Linnburn	TAIERI	Flathead	*G. depressiceps*	10
15	McPhees	TAIERI	Flathead	*G. depressiceps*	3
16	3 O'Clock Stream	TAIERI	Flathead	*G. depressiceps*	10
17	Canton Creek	TAIERI	Roundhead	*G. eldoni*	10
18	**Smugglers/Traquair**	TAIERI	Roundhead	*G. eldoni*	10
19	Shepherds Creek	TAIERI	Roundhead	*G. eldoni*	10
20	Suttons/Lee Stream	TAIERI	Roundhead	*G. eldoni*	10
21	Whare Creek	TAIERI	Roundhead	*G. eldoni*	10
22	**Munro Dam Creek**, Waipori	TAIERI	Roundhead	*G. pullus*	10
23	Crystal Creek, Waipori	TAIERI	Roundhead	*G. pullus*	10
24	**Ophir**, upper Manuherikia	CLUTHA	Roundhead	*G. anomalus*	10
25	Pomahaka tributary	CLUTHA	Flathead	Species D	10
26	Cardrona tributary	CLUTHA	Flathead	Species D	10
27	Boundary Creek, Lake Wanaka	CLUTHA	Koaro	*G. brevipinnis*	10
28	Poolburn Stream, Manuherikia R.	CLUTHA	Flathead	Species D	12
29	Poolburn, below dam, Manuherikia R.	CLUTHA	Flathead	Species D	4
30	Nevis River	CLUTHA	Roundhead	?*G.gollumoides*?	10
31	Walnut Creek, Lindis	CLUTHA	Flathead	Species D	9
32	L. Onslow tributary, Teviot River	CLUTHA	Flathead	Teviot	10
33	Omotu tributary	MATAURA	Roundhead	*G. gollumoides?*	10
34	Eyre Creek	MATAURA	Flathead	Southern	10
35	Mokoreta Stream	MATAURA	Flathead	Southern	2
36	Tributary at Athol	MATAURA	Roundhead	*G. gollumoides*	10
37	Weydon Burn	ORETI	Flathead	Southern	10
38	Tarwood Stream	CATLINS	Roundhead	*G. gollumoides*	9
39	Ourauea	WAIAU	Roundhead	*G. gollumoides*	10
40	**Chocolate Swamp**, Stewart Island	FRESHWATER	Roundhead	*G. gollumoides*	10
41	Freshwater River, Stewart Island	FRESHWATER	?Flathead?	?southern?	4
42	Rakeahua River (biv) Stewart Island	RAKEAHUA	Flathead	?southern?	2
43	Rakeahua River, Stewart Island	RAKEAHUA	?Flathead?	?southern?	8
44	Robertson River, Stewart Island	ROBERTSON	Roundhead	*G. gollumoides*	13

Measurements were undertaken generally with the use of either a low-power illuminated magnifier or, where dimensions were sufficiently small, using a low-power binocular microscope. All counts were taken using a low-power binocular microscope.

A total of 396 specimens were studied from 44 sites. Material examined is listed in Table 2, and names assigned to sample sites are based on the most recent taxonomy combined with distinctions derived from the genetic studies of Wallis, Waters and colleagues (cited in references). Where explicit identities of samples cannot be connected to described species, informal names are assigned to populations or groups of populations that are, in general, in current usage in published papers. Such names are enclosed within quotation marks, e.g. "species D" (from Waters et al. 1999; Esa et al. 2001; Waters & Wallis 2001a, 2001b).

Overall, the various non-migratory galaxiid populations in Otago and Southland appear to fall into two broad groupings which have become known as "flathead" and "roundhead" lineages. However, populations in and north of the Waitaki River, as well as some south of the Waitaki and east of the Taieri River (particularly in those river systems draining the Kakanui Mountains – Kakanui, Shag, Waianakarua Rivers) are not easily placed in either "flathead" or "roundhead" lineages). The various lineages, and our present understanding of their distributions are summarised below. Localities from which specimens were measured/counted are shown in **bold.**

The species/lineages include the following:

1. "northern" – comprises a group of populations encompassing sites in the upper Clarence (**Serpentine Stream)**, **Conway** and Wairau Rivers in Marlborough, the Maruia River (an upper tributary of the Buller River draining to the West Coast – **First Creek**), and probably also the headwaters of the **Motueka** River in inland Nelson (a population not yet examined genetically) (Waters & Wallis 2000).

2. *G. vulgaris* (sensu stricto) – populations across the Canterbury Plains from south of the Clarence River, including the type locality for this species in the Waimakariri River (**Rubicon River)** and south as far as the Waitaki River (**Maraewhenua River) (**Wallis et al. 2001).

3. *G. depressiceps* (= "flatheads") from the upper reaches of the Taieri River (**Linnburn, Nenthorn, McPhee,** and **3 O'clock Streams**, and **"Tributary A"** of the Kyeburn River**).** McDowall & Wallis (1996) included "flathead" stocks from the Clutha River system, and those draining the Southland Plains in *G. depressiceps*, but subsequent genetic studies (Waters & Wallis 2001a, 2001b; Waters et al. 2001a; Wallis et al. 2001) have suggested that several additional lineages occur in these areas, as discussed below; "flathead" fishes from the **Kakanui** and **Shag** were treated as "flatheads" in McDowall & Wallis (1996) but this identity is uncertain. Fish from the Shag tributary **McCormick's Creek** also had uncertain affinities based on molecular evidence (unpublished data).

4. "Species D" is a "flathead" lineage that genetic studies show to be distributed widely across the upper Clutha River system (**tributaries of the Lindis, Pomahaka, Cardrona,** and **Poolburn Rivers/Streams)** and downstream as far as at least Raes Junction**.** Populations of this complex in Totara Stream, a

tributary of the upper Taieri River that drains from the Rock and Pillar Range, have been shown to be hybrids between "species D" and *G. depressiceps* (Waters et al. 1999; Esa et al. 2001).

5. "teviot" comprises populations of "flathead" morphology in the Teviot River, which drains the southern flanks of the Lammerlaw Range into the Clutha River; genetic studies suggest that this is a distinct lineage (Waters & Wallis 2001b).

6. "southern" comprises lineages that are found in rivers that drain the Southland Plains including the Waiau, Mataura (**Mokoreta, Eyre**), Oreti (**Weydon Burn**), and Aparima Rivers, and also possibly several rivers in Stewart Island (**Rakeahua, Freshwater**) (Waters & Wallis 2001b; Waters et al. 2001b; Wallis et al. 2001).

7. *G. anomalus* is the "true" "roundhead", based on its first description among "roundhead" lineages (Stokell 1959; McDowall & Wallis 1996), and is found in upper tributaries of the Taieri River (**Healy, German)** as well as widely across the upper Manuherikia, a tributary of the Clutha River (**Ophir** – the type locality) (Allibone et al. 1996). Otherwise, "roundhead" lineages are somewhat surprisingly absent from the central/upper Clutha (but see next paragraph).

8. *G. gollumoides* is a "roundhead" morphotype that was described from Stewart Island (McDowall & Chadderton 1999), from both the **Robertson River** in the far south, and **Chocolate Swamp** in central Stewart Island. Genetic studies suggest that populations belonging to the "roundhead" lineage from across Southland (treated as belonging to *G. anomalus* in McDowall & Wallis 1996) belong to the same lineage as the Stewart Island "roundhead" populations, in the Waiau (**Orauia**), Mataura (**Omotu** and stream at **Athol**), Oreti, and Aparima Rivers, and also **Tarwood Stream**, a tributary of the Catlins River, in the Catlins area.

   A population that belongs to the *G. gollumoides* lineage is present in the **Nevis River**, which drains north into the Kawarau River, a Clutha River tributary. The Nevis is believed to have become connected to the Clutha owing to a river-capture event, having formerly flowed south to join the Mataura River (Waters et al. 2001b; Wallis et al. 2001). In addition, there are records of "roundheads" from tributaries of the Waiwera River, a south-bank lower tributary of the Clutha. These populations may need study, specifically to determine their affinities, whether with "roundheads" in the Taieri/ Manuherikia, or more probably to the Southland/Catlins stocks.

9. *G. eldoni* was described from tributaries of the lower Taieri (**Canton, Smugglers, Shepherds, Suttons, Whare** – McDowall 1997) and has since been found also in upper tributaries of Waipori, a southern Taieri River tributary (Allibone 1997, 1999), and in upper tributaries of the Tokomairiro River, an independent river system to the south of the Taieri. Genetic studies suggest that it is a "roundhead" lineage.

10. *G. pullus* is a further distinct species described from tributaries of the lower Waipori (McDowall 1997); it has since been found widely in the upper Waipori (Allibone 1997, 1999), and has spread west and south into upper tributaries of the Teviot, Tuapeka. Beaumont and Waitahuna Rivers – these being tributaries of the lower Clutha that drain the southern flanks of the Lammerlaw Ranges. Genetic evidence suggests that the fish from the type

locality of *G. pullus* (**Munro Dam Stream)** may be hybrids though this is apparently not true of the fish from **Crystal Creek**, nearby. Genetic studies also align these populations with "roundhead" lineages.

11. In addition to the *G. vulgaris* species complex lineages, data were taken from a sample of *G. brevipinnis* since it is accepted that the *G. vulgaris* species complex lineages are non-migratory derivatives from the diadromous *G. brevipinnis* (McDowall 1970, 1990; Waters & Wallis 2001b).

The above discussion provides some sense of the problems generated among the lineages of the *G. vugaris* species complex in the eastern South Island, and the incentive for studies that aim to clarify the taxonomy of the group and to identify characters that are useful in identifying specimens/populations of the various lineages. The scenario is highly complex, displays interesting concordances and conflicts with known geological events and scenarios (Waters & Wallis 2000; Waters et al. 2001b), and to make it all more complex there seem to be instances of hybridisation between lineages (Waters et al. 1999; Esa et al. 2001) that may or may not result from diversions of stream flows in association with late 19th Century alluvial gold mining in the Otago area. These complexities and uncertainties are the background to the present attempt to identify lineages and groupings of populations, and to discover characters useful in their identification.

3. Multivariate analysis of data

All analyses were carried out on data that were standardised to average length, to compensate for the fact that there were differences in the sizes of the fish measured among samples. These differences are regarded as an influence of sampling rather than a distinctive characteristic of the population studies (i.e. we have assumed that there are no systematic fundamental differences in absolute size among the populations sampled, that size, itself, is not a useful character for distinguishing populations). So, for all length/width measurements, the value of the characteristic was divided by the length of the animal, then multiplied by the average length found over all sites and species.

The significance of differences between groups, species or locations was assessed using a multivariate randomised permutation test on normalised Euclidean distances (ANOSIM – Clarke 1993, run in PRIMER – Clarke & Gorley 2001). Visualisations of differences were obtained using non-metric multi-dimensional scaling ordination plots (MDS – Clarke 1993, run in PRIMER– Clarke & Gorley 2001) or UPGAM (Unweighted Pair Group Method with Arithmetic Mean) trees. The MDS plots were based on average linkage clusters of normalised Euclidean distances. UPGMA (MOPED – Jowett 2001) is a straightforward method of tree construction. It uses a sequential clustering algorithm, in which local homology between OTUs (operational taxonomic units) is identified in order of similarity, and the tree is built in a stepwise manner using average-linkage clustering.

To determine the characteristics that distinguished between these two groups, discriminant analysis was used. Discriminant analysis helps to analyse the differences between groups and/or provide a means to assign (classify) any case or site into the group it most closely resembles. Thus it both investigates differences between groups and determines the most parsimonious way to distinguish among groups. Both the Mahalanobis and Euclidean distances were used initially; however, as similar results were obtained, the Mahalanobis distance was used thereafter. The characteristics for the discriminant analysis were selected using automatic stepwise procedures based on the ability of variables to discriminate between groups. The percentages of fish able to be correctly classified into 'species', using increasing numbers of characteristics, were calculated and presented for 90% and 95% correct classification.

4. Results

Two general morphotypes have been distinguished among the populations of Otago, Southland, and Stewart Island, commonly referred to as "roundheads" and "flatheads" for reasons that these names make obvious (references to McDowall, Wallis, Waters, and colleagues, listed below). Genetic and biogeograpical data suggest that populations to the north of Otago, i.e. from and including the Waitaki River, northwards, are derived from an Otago stock that 'escaped' from the Otago region and spread north across the Canterbury Plains as these were formed by erosion of the uplifting Southern Alps during the Pliocene and Pleistocene (Wallis et al. 2001). Populations of this species group in rivers draining the ranges of mountains to the east of the Taieri River and south of the Waitaki River valley—the Kakanui, Shag, and Waianakarua River systems—are equivocal in their identities and relationships among other populations of the species complex.

A series of analyses was undertaken designed to assist with clarifying whether a priori groupings could be distinguished using multivariate statistical methods. In addition we wished to determine whether we could identify explicit morphological characters, or groups of such characters, that could be used to distinguish groups of populations, and that could eventually be used to identify specimens from sites across the range of the *G. vulgaris* species group in the eastern South Island species group. The two general morphoptypes, "flatheads" and "roundheads", were dealt with somewhat separately during initial analyses, as discussed below.

1. Initially, differences in all measured variables between "roundheads" and "flatheads" were assessed using a multivariate randomised permutation test on normalised Euclidean distances. This analysis did not include fish from Kakanui, Shag (upper reaches of the river), and McCormick Creek (a lower tributary of the Shag River), because previous taxonomic and genetic work had produced equivocal identities and relationships of these populations to others of the group. In particular, the McCormick Creek population could be a hybrid stock (unpubl. data). There were significant differences between the

two morphotypes ($p = 0.001$), though a non-metric multidimensional scaling ordination plot showed considerable overlap between the two divisions.

Nine characteristics, listed below in order of the strength of their contribution to assisting separation of the lineages/species, were needed to separate "roundheads" from "flatheads" with a 90% correct classification (snout length; numbers of caudal fin rays; maximum length of anal fin; length lower jaw; eye diameter; head length; length caudal peduncle; number of anal fin rays; head width below eye). Thus, even attempts to separate what appeared to be distinctive morphological groups required a substantial (and impractical) number of characters to permit separation.

2. Despite the above result, it was decided to continue with this initial split and analyse the "roundhead" and "flathead" species/lineages separately, again without fish from Kakanui, Shag, and McCormick Creek.

    "Roundheads". Including "species D" as requested by DoC, all the roundhead lineages were significantly different from each other, based on all measured characteristics. However, seven characteristics (number of caudal fin rays; pyloric caeca and gill rakers; interorbital width; pectoral fin length; pelvic-anal length; length of upper jaw) were needed to separate lineages with a 90% correct classification. To get 95% of the fish correctly classified another 10 characteristics were needed (head width below eye; head length; maximum length of anal fin; pelvic fin length; number of dorsal and pectoral fin rays; standard length; length caudal peduncle; depth caudal peduncle; head depth).

    "Flatheads": Again including "species D" as requested by DOC, most flathead lineages were significantly different from each other, based on all measured characteristics. However, "species D" was not significantly different from *G. vulgaris* or "teviot". Fifteen characteristics (depth of gape; number of dorsal fin rays; preanal length; body depth at vent; postorbital length; pelvic fin length; head depth; number of pectoral fin rays; maximum length of dorsal fin; number of anal fin rays, width of gape; length of caudal peduncle; head width; interorbital width; predorsal length) were needed to separate the lineages with a 90% correct classification. To get 95% of the fish correctly classified another 7 characteristics were needed (pectoral-pelvic length; pelvic-anal length; length upper jaw; standard length; depth of caudal peduncle; eye diameter; head width below eye).

    These results highlight the difficulties encountered in identifying the different lineages from morphological characters.

3. Then data from Kakanui, Shag, and McCormick were added to the dataset and the "roundhead", "flathead" split was re-analysed. This increased the number of characteristics needed to correctly classify 90% of the fish to 11 (snout length; maximum length of anal fin; length of caudal peduncle; length of lower jaw; head width; body depth at vent; number of caudal fin rays; eye diameter; depth of gape; head length; number of pyloric caeca; and number of pectoral fin rays). Even including all measured characteristics, a 95% correct classification could not be achieved.

4. At this stage, as even the "roundhead"–"flathead" split was not clear, let alone the species within this split, it was decided to investigate whether locations differed in morphology. All locations had significantly different morphologies.

A large number of characteristics was required to separate out the localities (everything except length of the lower jaw). However, reasonable groupings (all localities except Freshwater and Poolburn 90% correctly classified) were obtained using 20 variables (number of caudal fin rays; number of pyloric caeca; body depth at vent; head width below eye; number of pectoral fin rays; eye diameter; pelvic fin length; length upper jaw; postorbital head length; interorbital width; head depth; pelvic-anal length; length of caudal peduncle; depth of gape; number of gill rakers; pectoral fin length; maximum length of anal; number of dorsal fin rays; standard length; and predorsal length). Again the number of characters needed to discriminate groups is large.

The various populations examined are numbered serially in Table 2 and Figure 1, to facilitate references in the following discussion.

Of particular significance, this analysis led to major differences in similarities compared with similarities of lineages generated using genetic data, as exemplified below:

Two populations of the genetically based "northern" lineage had reasonably close association in the tree (#2-**Conway** and **#22-Mariua**), but they were broadly separated from two others (#27-**Serpentine** and #37-**Motueka**). Fish from the Motueka are yet to be examined genetically, and their inclusion in "northern" is presently based on geographical distribution.

Populations of the *G. depressiceps* lineage in the upper Taieri River were also widely spread (#2-**Linnburn;** #11-**Nenthorn;** #16-**McPhee;** #18-**Tributary A;** #29-**3 O'clock**).

The two populations of *G. vulgaris* (sensu stricto) were also widely separated (#25-**Maraewhenua**, and #39-**Rubicon**).

Two populations of *G. pullus* were also far apart (#28-**Crystal;** #42-**Munro Dam Stream).**

"Species D" emerged widely across the clustering tree (#8-**Pomahaka Tributary;** #25 –**Cardrona;** #26-**Walnut Creek;** Lindis River; #40-**Poolburn Dam Stream;** #41-**Poolburn Stream).**

Thus, the analysis of data separately by population resulted in major fragmentation of what genetic data suggest are lineages, with virtually all major lineages tending to be broadly dispersed across a tree based on morphology.

5. An analysis was undertaken of the two more northern lineages, since these tend to be quite separate, geographically, from the highly complex lineages of Otago and Southland. This entailed addressing separation of *G. vulgaris* (sensu stricto) – **Maraewhenua** and **Rubicon**, and "northern" (**Clarence - Serpentine**, **Maruia**, **Conway** and **Motueka** data only. Only six characteristics (pelvic fin length; number of anal fin rays; body depth at vent; depth of gape; width of gape; head depth) were needed to separate the lineages with a 90% correct classification. Actually this resulted in 100% classification for all but **Maruia** and **Serpentine**. To get 95% of the fish correctly classified only another 2 characteristics (head width; eye diameter) were needed. This resulted in one **Maruia** fish being classified as **Serpentine** and one **Serpentine** being classified as **Maruia**. Using snout length, dorsal fin rays, pectoral-pelvic length and predorsal length, as well, resulted in only one fish being misclassified (as **Serpentine** rather than **Maruia**).

UPGMA tree

Sample #		Genetic lineage	Serial #
BoundaryCr	27	*G. brevipinnis*	1
Linnburn	14	*G. depressiceps*	2
Weydon	37	**Southern**	3
Kakanui	7	**?**	4
Nenthorn	13	*G. depressiceps*	5
Mokoreta	35	**Southern**	6
Pigroot	8	*G. eldoni*	7
PomaTrib	25	**Species D**	8
German	11	*G. anomalus*	9
Canton	17	*G. eldoni*	10
* Smugglers	18	*G. eldoni*	11
SuttonsLee	20	*G. eldoni*	12
Orauea	29	**Orauea**	13
3Oclock	16	*G. depressiceps*	14
Shepherds	19	*G. eldoni*	15
Biv	42	*G. gollumoides*	16
Freshwater	41	**Southern**	17
Nevis	30	*G. gollumoides*	18
Healy	10	*G. anomalus*	19
Ophir	24	*G. anomalus*	20
Omotu	33	*G. gollumoides*	21
Tarwood	38	*G. gollumoides*	22
McPhee	15	*G. depressiceps*	23
EyreCreek	34	**Southern**	24
Maraewhenua	6	*G. vulgaris*	25
Maruia	4	**Northern**	26
Serpentine	2	**Northern**	27
Crystal	23	*G. pullus*	28
Conway	3	**Northern**	29
Cardrona	26	**Species D**	30
WalnutLindis	31	**Species D**	31
* Triba	12	*G. depressiceps*	32
Teviot	32	**Teviot**	33
McCormick	9	**?**	34
Athol	36	*G. gollumoides*	35
Whare	21	*G. eldoni*	36
Motueka	1	**Northern**	37
Pooldam	29	**Species D**	38
* Rubicon	5	*G. vulgaris*	39
Poolburn	28	**Species D**	40
* Munro	22	*G. pullus*	41
Rakeahua	43	**Southern**	42
Robertson	44	*G. gollumoides*	43
* ChocSwamp	40	*G. gollumoides*	44

0   Linkage distance   1

* − type locality

Figure 1. Clustering tree based on morphological similarity (serial numbers in right column to facilitate reference).

So pelvic fin length, number of anal fin rays, body depth at vent, depth of gape, width of gape, and head depth, separate out **Maraewhenua**, **Rubicon** and **Conway** from each other and the remaining three. Head width separates **Motueka** from Clarence/**Serpentine** and **Maruia**. And eye diameter can differentiate between most of the Clarence/**Serpentine** and **Maruia** fish.

Of particular interest, the six characters listed above that allowed 100% separation of all but **Maruia** and **Serpentine** therefore permitted complete separation of the "northern" and *G. vulgaris* lineages, since **Maruia** and **Serpentine** both belong to the "northern" lineage. Even so, that six characters were needed provides a considerable barrier to field identification.

One interesting aspect of the question relates to the ability to distinguish *G. pullus* and *G. eldoni* from each other and from all other lineages. Both populations of *G. pullus* have a highly distinctive number of principal caudal rays (14 rays), as do the populations of *G. eldoni* (15); all other populations having 16 caudal rays, which is a very stable count found widely across the family Galaxiidae (see Table 3, derived from McDowall 2001)

This character, alone, ought to have allowed both strong separation of the members of each lineage from all other populations, and their emergence in the tree close together. But, as noted above, the two populations of *G. pullus* were widely separated (#27 & #42). Three of the *G. eldoni* populations were placed close together (#6-**Canton;** #7-**Smugglers;** #8-**Suttons/Lee Stream**), but fourth and fifth populations were well distant #28-**Whare**, and #30-**Shepherds**).

There are substantial separations evident among populations within these two lineages despite the unusual similarity within each, in having deviant principal

TABLE 3.   VARIATION IN CAUDAL FIN RAY COUNTS IN NEW ZEALAND SPECIES OF *GALAXIAS*.

Modal counts that differ from the normal modal counts for most *Galaxias* species are shown in bold. Data from McDowall & Waters (2002).

| SPECIES | CAUDAL FIN RAYS | | | | | |
	13	14	15	16	17	18
G. anomalus	-	-	-	39	1	-
G. argenteus	-	-	1	38	-	-
G. brevipinnis	-	-	5	206	3	1
G. cobitinis	-	-	**15**	-	-	-
G. depressiceps	-	-	4	92	4	-
G. divergens	1	3	**83**	6	-	-
G. eldoni	-	4	**45**	1	-	-
G. fasciatus	-	-	1	60	2	-
G. gollumoides	-	-	3	20	-	-
G. gracilis	-	-	4	42	4	-
G. maculatus	-	-	1	80	-	-
G. paucispondylus	-	-	3	54	1	-
G. postvectis	-	-	-	25	-	-
G. prognathus	-	-	1	30	4	-
G. pullus	1	**39**	1	1	-	-
G. rekohua	-	-	-	11	-	-
G. vulgaris	—	-	4	99	5	1

caudal fin ray counts. These separations are driven by other morphological differences among populations within each lineage, combined with similarities between populations within the lineages to other populations.

5. Discussion

Cluster analysis (using UPGMA) was applied first to populations grouped according to the species or lineages recognised from the published taxonomic and molecular studies, and this analysis showed that there are groups of characters that can be used to distinguish these species/lineages; however, the clustering was generated by substantial numbers of characters and isolated no single characters that are generally useful for identification of lineages. Moreover, the ability to distinguish the lineages has to be regarded as a 'statistical ability' rather than a practical approach, especially where there is an interest in using such characters to enable identification of the lineages in the field.

The same approach to analysis was applied at the population, rather than species or lineage level, to see how cluster analysis grouped the various populations without any *a priori* attempt to group populations into lineages or recognised taxa. At this point, the analysis failed to group the various species/ lineages either by the molecular data, nor by formally described species. Thus morphological information performs poorly at grouping taxa/lineages into the groups indicated from past taxonomic/molecular studies. Given that this is so, the analysis discussed above clearly seems to be more fortuitous than useful.

Thus the outcome of the search for morphological characters that are helpful in sorting and identification of samples or individuals is essentially a failure. Present morphological information does not permit such identification, which appears to depend much more on molecular data. Decisions need to be made, in the light of the present results and the molecular data, on which lineages, and in what groupings, species are formally recognised.

6. Acknowledgments

7. References

Allibone, R.M. 1997: Freshwater fish of the Otago region. *Otago Conservancy Miscellaneous Report Series* 36: 1–54.

Allibone, R.M. 1999: Impoundment and introductions: their impacts on native fish of the upper Waipori River, New Zealand. *Journal of the Royal Society of New Zealand* 29: 291–299.

Allibone, R.M.; Crowl, T.A.; Holmes, J.M.; King, T.M.; McDowall, R.M.; Townsend, C.R.; Wallis, G.P. 1996: Isozyme analysis of *Galaxias* species (Teleostei: Galaxiidae) from the Taieri River, South Island, New Zealand: a species complex revealed. *Biological Journal of the Linnean Society* 57: 107–127.

Clarke, K.R. 1993: Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Clarke, K.R.; Gorley, R.N. 2001: Primer v. 5. Primer-E, Plymouth.

Esa, Y.B.; Waters, J.M.; Wallis, G.P. 2001: Introgressive hybridization between *Galaxias depressiceps* and *Galaxias* sp. D (Teleostei: Galaxiidae) in Otago, New Zealand: secondary contact mediated by water races. *Conservation Genetics* 1: 329–339.

Jowett, I.G. 2001: *Modelling patterns in environmental data, Version 1:01.* NIWA, Hamilton.

McDowall, R.M. 1970: The galaxiid fishes of New Zealand. *Bulletin of the Museum of Comparative Zoology, Harvard University* 139: 341–431.

McDowall, R.M. 1990: New Zealand freshwater fishes: a natural history and guide. Heinemann Reed, Auckland.

McDowall, R.M. 1997: Two further new species of *Galaxias* (Teleostei: Galaxiidae) from the Taieri River, southern New Zealand. *Journal of the Royal Society of New Zealand* 27: 199–217.

McDowall, R.M. 2001: The principal caudal fin ray count – a fundamental character in the galaxioid fishes. *New Zealand Journal of Zoology 28*: 395–405.

McDowall, R.M.; Chadderton, W.L. 1999: *Galaxias gollumoides* (Teleostei: Galaxiidae), a new fish species from Stewart Island, with notes on other non-migratory freshwater fishes present on the island. *Journal of the Royal Society of New Zealand* 29: 77–88.

McDowall, R.M.; Wallis, G.P. 1996: Description and redescription of *Galaxias* species (Teleostei: Galaxiidae) from Otago and Southland. *Journal of the Royal Society of New Zealand* 26: 401–427.

McDowall, R.M.; Waters, J.M. 2002: A new longjaw galaxias species (Teleostei: Galaxiidae) from the Kauru River, North Otago, New Zealand. *New Zealand Journal of Zoology 29*: 41–52.

Stokell, G. 1959: Notes on galaxiids and eleotrids with descriptions of new species. *Transactions of the Royal Society of New Zealand 82*: 411–418.

Wallis, G.P.; Judge, K.F.; Bland, J.; Waters, J.M.; Berra, T.M. 2001: Genetic diversity in New Zealand *Galaxias vulgaris* sensu lato (Teleostei: Osmeriformes: Galaxiidae): a test of a biogeographic hypothesis. *Journal of Biogeography* 28: 59–67.

Waters, J.M.; Wallis, G.P. 2000: Across the Southern Alps by river capture? Freshwater fish phylogeography in South Island, New Zealand. *Molecular Ecology* 9: 1577–1582.

Waters, J.M.; Wallis, G.P. 2001a: Mitochondrial DNA phylogenetics of the *Galaxias vulgaris* complex from South Island, New Zealand: rapid radiation of a species flock. *Journal of Fish Biology* 58: 1166–1180.

Waters, J.M.; Wallis, G.P. 2001b: Cladogenesis and loss of the marine life-history phase in freshwater galaxiid fishes (Osmeriformes: Galaxiidae). *Evolution* 55: 587–597.

Waters, J.M.; Esa, Y.B.; Wallis, G.P. 1999: Characterization of microsaltellite loci from a New Zealand freshwater fish (*Galaxias vulgaris*) and their potential for analysis of hybridization in Galaxiidae. *Molecular Ecology* 8: 1080–1082.

Waters, J.M.; Esa, Y.B.; Wallis, G.P. 2001a: Genetic and morphological evidence for reproductive isolation between sympatric populations of *Galaxias* (Teleostei: Galaxiidae) in South Island, New Zealand. *Biological Journal of the Linnean Society* 73: 287–298.

Waters, J.M.; Craw, D.; Youngson, J.H.; Wallis, G.P. 2001b: Genes meet geology: fish phylogeographic pattern reflects ancient rather than modern drainage patterns. *Evolution* 55: 1844–1985.