Genetic analyses of carp, goldfish, and carp–goldfish hybrids in New Zealand

DOC RESEARCH & DEVELOPMENT SERIES 219

P.J. Smith and S.M. McVeagh

Published by
Science & Technical Publishing
Department of Conservation
PO Box 10–420
Wellington, New Zealand

DOC Research & Development Series is a published record of scientific research carried out, or advice given, by Department of Conservation staff or external contractors funded by DOC. It comprises reports and short communications that are peer-reviewed.
Individual contributions to the series are first released on the departmental website in pdf form. Hardcopy is printed, bound, and distributed at regular intervals. Titles are also listed in our catalogue on the website, refer http://www.doc.govt.nz under Publications, then Science and research.

ISSN 1176-8886
ISBN 0-478-14026-6

This report was prepared for publication by Science & Technical Publishing Section; editing and layout by Geoff Gregory. Publication was approved by the Chief Scientist (Research, Development & Improvement Division), Department of Conservation, Wellington, New Zealand.

CONTENTS

Genetic analyses of carp, goldfish, and carp–goldfish hybrids in New Zealand

P.J. Smith and S.M. McVeagh

National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington, New Zealand

ABSTRACT

To identify strains of carp *Cyprinus carpio* and suspected hybrids with goldfish *Carassius auratus,* genetic markers were tested in fish samples in populations from Canterbury, Nelson, Waikato, Whangarei, and the lower North Island of New Zealand. Carp–goldfish hybrids were identified in samples from the Waikato River and Whangamarino swamp, central North Island; one hybrid was found in the Nelson area. Based on mitochondrial DNA sequences, hybridisation occurred with either species as the female parent. Mitochondrial DNA sequencing and use of 'restriction enzymes' were tested on carp strains and tissue samples of goldfish from New Zealand, together with Crucian carp *Carassius carassius* from Germany and the UK, and carp strains from Australia, Germany, the UK, and Vietnam. Carp from New Zealand had different cytochrome *b* sequences, and a different restriction fragment profile, from carp from the European and Vietnamese sources. There was no evidence for the presence of carp from Europe or Vietnam or Crucian carp in the New Zealand samples. Cytochrome *b* sequences of carp from Australia aligned with those from Europe, except for one specimen from Lake Burley Griffin, which aligned with carp from New Zealand. This excluded the Australian Boolara strain from being a source of carp in New Zealand, but did not allow conclusions as to whether koi in New Zealand were derived from Australia or directly from Asia. The goldfish in New Zealand aligned with *Carassius auratus* from the UK.

Keywords: carp, *Cyprinus carpio*, goldfish, *Carassius auratus*, intergeneric hybrids, genetic provenance, allozymes, mitochondrial DNA, New Zealand.

Smith, P.J.; McVeagh, S.M. 2005: Genetic analyses of carp, goldfish, and carp–goldfish hybrids in New Zealand. *DOC Research & Development Series 219*. Department of Conservation, Wellington. 20 p.

1. Introduction

Over the past few years new populations of the coarse fish carp *Cyprinus carpio*, goldfish *Carassius auratus*, and rudd *Scardinius erythropthalmus* have been discovered in both the North and South Islands of New Zealand. These fish are appearing outside previously recorded ranges, and human-mediated introductions are the most likely cause. Source areas for these introductions are unknown, but it is likely that they have been derived from established New Zealand populations. However, there were concerns that carp may have been introduced into the South Island from Australia (L. Chadderton pers. comm.). It has even been suggested that the Crucian carp *Carassius carassius* might have been inadvertently introduced and released with goldfish. *Carassius carassius* is often confused with *C. auratus* in the UK and forms hybrids with both *C. carpio* and *C. auratus* (Wheeler 2000).

Fish survey work by Department of Conservation (DOC) staff has located populations of carp and goldfish in new areas, especially in Canterbury, and some fish display morphological characteristics of hybrids between carp and goldfish, although the fish have not been conclusively identified. Populations of carp and goldfish in other regions have also shown unexpected population increases and changes in behaviour (L. Chadderton pers. comm.). It is unknown whether fish with ambiguous morphological and/or population characteristics are carp, goldfish, carp–goldfish hybrids, or a novel carp stock previously unknown in New Zealand.

Early genetic work on carp in New Zealand recorded carp–goldfish hybrids from Tauranga, the Whangamarino Stream, and Waikato River, and demonstrated genetic and morphological characters that distinguish F_1 hybrids from the parent species (Pullan & Smith 1987). These hybrids appeared similar to feral goldfish but possessed one pair of small barbels and showed both carp and goldfish alleles at diagnostic allozyme loci (Pullan & Smith 1987).

This investigation was undertaken to test genetic markers in carp and goldfish populations in New Zealand, and to identify suspected hybrid fish and the carp strain(s) in samples from populations from Canterbury, Waikato, and other locations in New Zealand. Allozyme identification was the method used to detect hybrids and mitochondrial (mt)DNA was used for identification of the carp strains. Analyses were undertaken in 2002/03. Additional carp samples from Australia and Vietnam were supplied in 2004 and sequenced for two regions of mtDNA.

2. Methods

2.1 SPECIMENS AND TISSUE COLLECTION

Whole frozen carp, goldfish, and suspected hybrids were sent to the National Institute of Water & Atmospheric Research (NIWA), Wellington, by DOC staff. Specimen localities are summarised in Table 1. A small piece of white muscle tissue was removed from each specimen, and the specimen and corresponding tissue sample numbered with the same code number. Whole specimens were re-frozen and forwarded to Dr R.M. McDowall, NIWA, Christchurch, for morphological analyses. Specimens labelled as carp had the typical orange coloration of koi carp and barbels around the mouth, while goldfish were either orange or olive brown and lacked barbels. Tissue samples were stored at –70°C. Liver and heart tissues were not routinely removed because several specimens

TABLE 1. SOURCES OF NEW ZEALAND SAMPLES OF CARP, GOLDFISH, AND SUSPECTED HYBRIDS, AND RESULTS OF ALLOZYME ANALYSES.

AREA	LOCATION	RESULT
Nelson	Deaker property	1 carp; 1 hybrid; 1 rudd
	Flounders Park	4 carp
	Blue Creek	1 goldfish
	Stringers Pond	1 carp
	Queens garden	2 carp
Motueka	Moutere Stream	1 carp; 7 goldfish
Canterbury	Garden City	2 goldfish
	Halswell River	2 goldfish
	Timaru	3 goldfish
	Akaroa	2 goldfish
	Kaiapoi	3 goldfish
	Otipua Wetlands	3 goldfish
	Saltwater Creek	1 goldfish
	ABG1	1 carp
Whangarei	Trumain Road	1 goldfish
	Mill Brook Road	1 carp
Waikato	Ohinimuri koi pond	17 carp
	Lake Whangape	10 carp
	North, no specific site	10 goldfish
	Various	9 carp; 3 hybrids
	Whangamarino	9 hybrids
Dannevirke	Johansens Pond	1 carp
Wanganui	Sicely Pond	4 carp
Wairarapa	Whakarewa Stream	1 goldfish

had been stored in domestic freezers for one or more years and it is likely that these tissues would be in poor condition for allozyme tests.

Muscle tissue samples were obtained from carp, goldfish, and Crucian carp from Germany and the UK, and carp from New South Wales in 2002/03. Additional carp muscle tissue samples from Australia (ACT, New South Wales, Tasmania, and Victoria) and Vietnam were obtained in 2004 (Table 2), for use as controls in the mtDNA analyses. Muscle tissues were preserved in ethanol (and were not suitable for allozyme analyses).

Whole frozen specimens have been sent to Dr R.W. McDowall, NIWA Christchurch, for morphological study. Frozen muscle tissue samples from carp, goldfish, and hybrids from New Zealand are stored at NIWA Wellington, as are ethanol-fixed muscle tissue samples from carp from Australia, Europe, and Asia and goldfish from Europe.

2.2 DETECTION OF HYBRIDS

2.2.1 Allozymes

Electrophoretic procedures followed those described in Pullan & Smith (1987) and Benson & Smith (1989) for cellulose acetate gels. Six enzymes were tested in 16 fish, selected from the Waikato, Canterbury, and Nelson samples: glycerol-3-phosphate dehydrogenase (Enzyme Commission Number 1.1.1.8), glucose-6-phosphate isomerase (5.3.1.9), lactate dehydrogenase (1.1.1.27), malate dehydrogenase (1.1.1.37), malic dehydrogenase (1.1.1.40), and phospho-glucomutase (5.4.2.2). Because of inconsistent resolution of the other enzymes in some tissue samples, only two enzymes, lactate dehydrogenase (LDH) and malate dehydrogenase (MDH), were routinely tested in all specimens. These muscle-specific loci LDH and MDH were reported to be monomorphic (showed no genetic variation) in population samples of wild and domesticated carp from Japan, Germany, Russia, and Israel, while other allozyme markers showed genetic variation within and among European and Asian carp (Kohlman & Kersten 1999).

2.2.2 Microsatellite DNA

Following the allzoyme analyses a few selected specimens of carp, goldfish, and carp–goldfish hybrids were sent to Dr Bernd Haenfling, Molecular Ecology and Fisheries Genetics, Department of Biological Sciences, University of Hull, UK for testing with five microsatellite markers developed for carp. The microsatellite markers have diagnostic alleles for carp, goldfish, and Crucian carp (B. Haenfling pers. comm.). The results are presented here with permission of Dr Haenfling.

TABLE 2. CONTROL SAMPLES USED FOR MITOCHONDRIAL DNA ANALYSES.

SPECIES	NO. OF SPECIMENS	LOCATION	SOURCE
Carp *Cyprinus carpio*	1	Elbe, Germany	B. Haenfling
Carp *Cyprinus carpio*	1	Danube, Germany	B. Haenfling
Carp *Cyprinus carpio*	9	Lake Liddell, NSW	M. Lowry
Carp *Cyprinus carpio*	1	Tasmania	B. Tucker
Carp *Cyprinus carpio*	2	Gippsland, Victoria	B. Tucker
Carp *Cyprinus carpio*	1	Lake Burley Griffin, ACT	B. Tucker
Carp *Cyprinus carpio*	3	Narrandera, NSW	B. Tucker
Carp *Cyprinus carpio*	5	Vietnam	K. Kohlmann
Carp *Cyprinus carpio*	1	UK	D. Smallwood
Crucian carp *Carassius carassius*	1	UK	D. Smallwood
Crucian carp *Carassius carassius*	2	Elbe, Germany	B. Haenfling
Goldfish *Carassius gibelio*	1	Elbe, Germany	B. Haenfling
Goldfish *Carassius auratus*	1	UK	D. Smallwood

2.3 IDENTIFICATION OF CARP STRAINS

2.3.1 Mitochondrial DNA analyses

DNA was extracted from selected koi carp and goldfish from New Zealand sites. Genuine F_1 hybrids, identified with allozyme analyses, were included in the mtDNA analyses. Samples of carp from Australia, Germany, and the UK; goldfish from Germany and the UK; and Crucian carp from Germany and the UK were used as controls (Table 2).

The mitochondrial analyses were undertaken to identify the strain of carp in New Zealand, and to test for the presence of Crucian carp. In addition, because mtDNA is maternally inherited, only the female parent is represented in the hybrid, thus providing a test of the direction of hybridisation (i.e. whether the female parent is a carp or goldfish).

Total genomic DNA was extracted from 200–500 mg of muscle tissue by homogenisation and digestion with proteinase-K at 55°C for 4 h. After digestion DNA was extracted with phenol:chloroform, followed by chloroform:isoamyl alcohol, and precipitated with 70% ethanol at –20°C, adapted from Taggart et al. (1992). The DNA pellet was air-dried and re-suspended in 40 µl sterile water and stored at –20°C.

2.3.2 Mitochondrial DNA sequencing and data analysis

Two regions of the mitochondrial genome were amplified by the polymerase chain reaction (PCR) in 50 ml volumes in a Cetus DNA thermocycler (Perkin-Elmer Corporation, Connecticut). The primer pair Cyb 2 and tGludg (Palumbi et al. 1991), that amplify an approximate 500 base pair region of the cytochrome *b* gene in fish (Baker et al. 1995), and the primer pair CRH00 and CRL47L (Inoue et al. 2001), that amplify an approximate 700 base pair region of the control region (the 5´ end), were used with all specimens. Amplifications for cytochrome *b* were carried out using an initial denaturation of 94°C for 2 min;

34 cycles of 92°C for 60 s, 54°C for 60 s, and 72°C for 90 s, followed by an extension at 72°C for 8 min. For the control region, amplifications were carried out using an initial denaturation of 94°C for 2 min; 35 cycles of 94°C for 60 s, 54°C for 60 s, and 72°C for 120 s, followed by an extension at 72°C for 7 min. Amplified products were separated in 1.4% agarose gels in a TBE buffer (25 m*M* Tris, 0.5 m*M* EDTA, and 25 m*M* boric acid), stained with ethidium bromide, and viewed under ultraviolet light. Those samples producing a single DNA fragment in the agarose gel were purified using the QIAquick gel extraction kit (Qiagen). Sequences were determined using the ABI Taq DyeDeoxy™ Terminator Cycle Sequencing Kit according to the manufacturer's directions (Applied Biosystems Inc.) and run on an ABI prism autosequencer.

Sequences were edited in CHROMAS (Technelysium, Queensland), and aligned in the BIOEDIT programme (Hall 1999). The cytochrome *b* and control region sequences were aligned against the complete mtDNA sequence for common carp *C. carpio* (GenBank Accession No. X61010), and other partial sequences for *C. carpio* (AU301083), *Carassius auratus* (AF045966 and AF051858) and *C. auratus langsdorfi* (AB006953). Rudd *Scardinius erythropthalmus* and *S. acarnanicus* (data from GenBank Accession Nos. Y104444 and AF090775 respectively) were used to root the cytochrome *b* trees.

Phylogenetic analyses were performed separately for the cytochrome *b* and control region sequences. The control region is non-coding and subject to different evolutionary constraints from the cytochrome *b* gene (McCune & Lovejoy 1998). Initial phylogenies were explored with PAUP version 4.0 (Swofford 2000) using three different optimality criteria (maximum parsimony, minimum evolution, and maximum likelihood). Parsimony analyses were performed using heuristic searches, employing tree bisection-reconnection branch swapping. Minimum evolution was based on LogDet (Lockhart et al. 1994) genetic distances. Modeltest version 3.06 (Posada & Crandall 1998) was used to determine the best-fit model using likelihood ratio tests. Neighbour-joining trees were constructed based on the Tamura & Nei (1993) model for control region sequences, and the general time-reversible (GTR) model (Rodriquez et al. 1990) for cytochrome *b* sequences. Support for each internode was evaluated by bootstrap replications (Felsenstein 1985).

2.3.3 Restriction fragment length polymorphism analysis

Selected specimens of carp from all regions of New Zealand, along with carp control samples from Germany, New South Wales, and the UK were tested with the restriction fragment length polymorphism (RFLP) method of analysing mtDNA, which uses restriction enzymes to cut specific amplified regions of mtDNA into fragments, which are then separated by size in a gel medium.

The NADH-3,4 dehydrogenase (ND-3/4) region of mtDNA was amplified with the primer pair ND3/4a and ND3/4b designed by Chang et al. (1994) for carp. Amplification protocols followed those described above for mtDNA sequences. Amplified products were digested with carp-strain diagnostic restriction enzymes (described by Gross et al. 2002) in 20 ml volumes for a minimum of four hours, following manufacturer's recommendations (New England BioLabs, Beverly, MA). The digested products were separated in 1.2% agarose gels and run at 60V for 1.5–2 hours. The amplified products were detected with

ethidium bromide, which had been added to the gel, and viewed under ultraviolet light. The diagnostic restriction enzymes were *Alu I*, *Hapa II*, and *Hinf I*, which produce different haplotypes in European and Asian carp (Gross et al. 2002).

3. Results

3.1 DETECTION OF HYBRIDS

3.1.1 Allozymes

The two enzymes, LDH and MDH, resolved well in all muscle tissue samples. They both have different alleles in carp and goldfish (Pullan & Smith 1987), and these species differences were confirmed by analysis of the control samples: carp had a slow migrating band for both LDH and MDH, while goldfish had a fast migrating band for both LDH and MDH. Hybrids appeared with both slow and fast migrating bands at both loci.

Carp–goldfish hybrids were found in the Waikato River and Whangamarino swamp in the central North Island. One hybrid was found in the Nelson area. Two unusual specimens from Whangarei were a carp and a goldfish, and not hybrids. One specimen from Nelson produced atypical gel phenotypes for both LDH and MDH, with an intermediate band pattern for MDH and an extra slow band for LDH, compared with the control samples from carp and goldfish. This specimen was identified as a rudd with the mtDNA control region and cytochrome *b* sequence analyses.

Results are summarised in Table 1. Full details are available from the authors.

3.1.2 Microsatellite DNA

Five suspected hybrids, identified with allozyme markers, were confirmed as carp–goldfish hybrids, using the microsatellite markers.

3.2 IDENTIFICATION OF CARP STRAINS

3.2.1 Mitochondrial DNA sequences

Sequence data were collected for 600 bases of control region and for 409 bases of cytochrome *b* in specimens of carp, goldfish, and carp–goldfish hybrids from New Zealand, and compared with corresponding sequences from control samples, and from GenBank. Seventy-two of the control region sites were parsimony informative and there was a 0.010 ± 0.003 sequence divergence among the carp and 0.008 ± 0.002 divergence among the goldfish (0.0 excluding *Carassius gibelio*). Likewise, 43 of the cytochrome *b* sites were parsimony informative and there was a 0.012 ± 0.004 sequence divergence among the carp and 0.009 ± 0.003 divergence among the goldfish (0.004 ± 0.002 excluding *C. gibelio*).

New Zealand specimens appeared as either carp or goldfish, there was no evidence for Crucian carp in any of the samples tested. Cytochrome *b* sequences in New Zealand goldfish differed from the UK goldfish sequence by 2–3 base substitutions, and from *C. gibelio* by 7 base substitutions (Fig. 1A).

The cytochrome *b* sequences showed four clusters of carp with strong bootstrap support (> 70%). One cluster included control samples from Germany, New South Wales, Tasmania, Victoria, and the UK. A second cluster included all the New Zealand specimens and one specimen from Lake Burley Griffin, ACT (Fig. 1A). New Zealand carp differed from the European (German/UK/Australia, excluding ACT) carp by 6–7 base substitutions. Two specimens from GenBank (presumably from Asia, based on authors' addresses) formed a

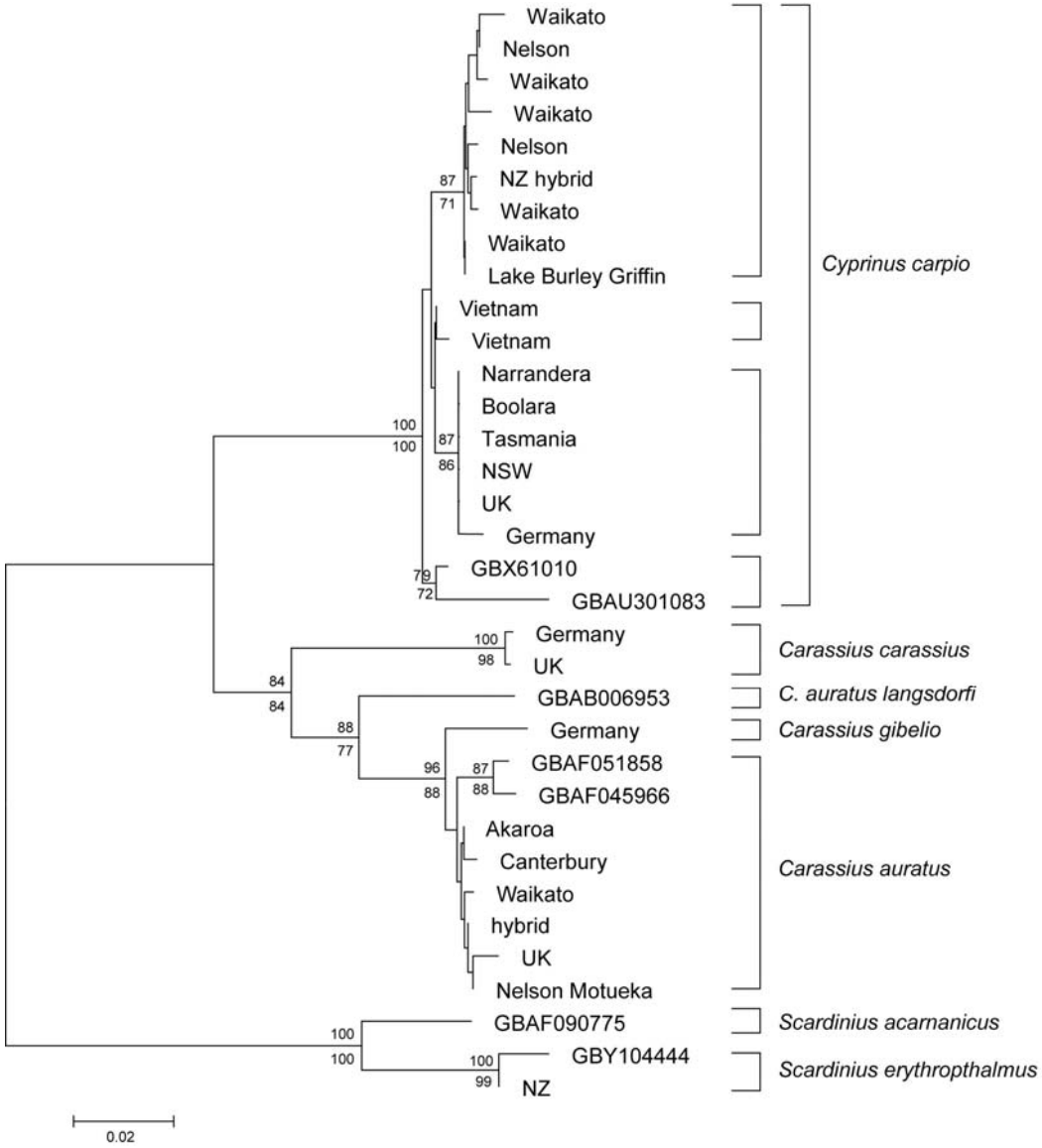

Figure 1A. Phylogenetic relationships of carp from Australia and Vietnam, and carp and goldfish specimens from New Zealand, Germany and the UK based on cytochrome *b* sequences. Scale bars represent an interval of the general time-reversible model (Rodriquez et al. 1990), and numbers at nodes are bootstrap percentages (1000 replicates) based on distance (above nodes) and parsimony (below nodes). Trees were rooted with the outgroup rudd *Scardinius erythropthalmus* from New Zealand and GenBank and *Scardinius acarnanicus* from GenBank. Control samples are goldfish *Carassius gibelio* and *C. auratus* from Germany and the UK, respectively; carp *Cyprinus carpio*: from Australia, Germany, UK, and Vietnam; and Crucian carp *Carassius carassius* from Germany and the UK. Code numbers refer to GenBank (GB) Accession numbers.

third well-supported cluster. The Vietnamese samples produced a fourth cluster (Fig. 1A.). It is possible that the GenBank specimens were Amur carp, which differ from koi carp (Kohlmann and Kersten 1999; Gross et al. 2002). Unpublished carp cytochrome *b* sequences in GenBank (Zhou et al. 2003a) were included in a second phylogenetic analysis (Fig. 1B). Assuming that the specimen codes were equivalent to those in Zhou et al. (2003b), the Volga River and German mirror carp form a separate well supported cluster to the European/Australian carp (Fig. 1B). The Asian carp sequences (Accession Numbers AY3472xx) cluster with two other sequences from GenBank (Fig. 1B).

The carp control region sequences showed sequence variation, but no clear geographical differentiation among the European, Australian, New Zealand and Vietnamese specimens (Fig. 2). Control region sequences in the goldfish from New Zealand were identical to those from the UK *Carassius auratus* control sequence.

Hybrids tested with the mtDNA sequences appeared as either carp or goldfish demonstrating that hybridisation takes place in both directions (i.e. with female goldfish or female carp).

3.2.2 Restriction fragment length polymorphism analysis

The restriction enzymes *Hinf I* and *Hpa II* both produced two different restriction digest fragment profiles of the ND-3/4 region: one in carp from Germany, New South Wales, and the UK; and a second in all the carp tested from New Zealand. No European carp digestion profiles were found in the New Zealand carp specimens. A third restriction enzyme, *Alu 1*, produced identical fragment haplotypes in all specimens, and did not distinguish between carp from Germany, NSW, and the UK, and carp from New Zealand.

4. Discussion and conclusions

4.1 CARP–GOLDFISH HYBRIDS

Carp–goldfish hybrids were found in the Waikato and the Whangamarino swamp, areas where hybrids were first reported in 1987 (Pullan & Smith 1987). One additional carp–goldfish hybrid was found in the Nelson area. Hybrids had also been reported from Tauranga (Pullan & Smith 1987), so hybridisation appears to be widespread in New Zealand. The specific conditions which lead to hybridisation are unclear, but mtDNA sequence data indicate that either species can act as the female parent. The hybrids all appeared to be first-generation crosses with simple combinations of carp and goldfish alleles in both this and the previous study (Pullan & Smith 1987). Chromosomal studies of carp–goldfish hybrids in Russia found the hybrids to be triploids (Zelinskij et al. 1992), and by implication the hybrids would be sterile. However, Hume et al. (1983) reported spent male and female carp–goldfish hybrids in Victoria, Australia, indicating that hybrids are reproductively active, although this does not necessarily mean that eggs or sperms or any offspring are viable.

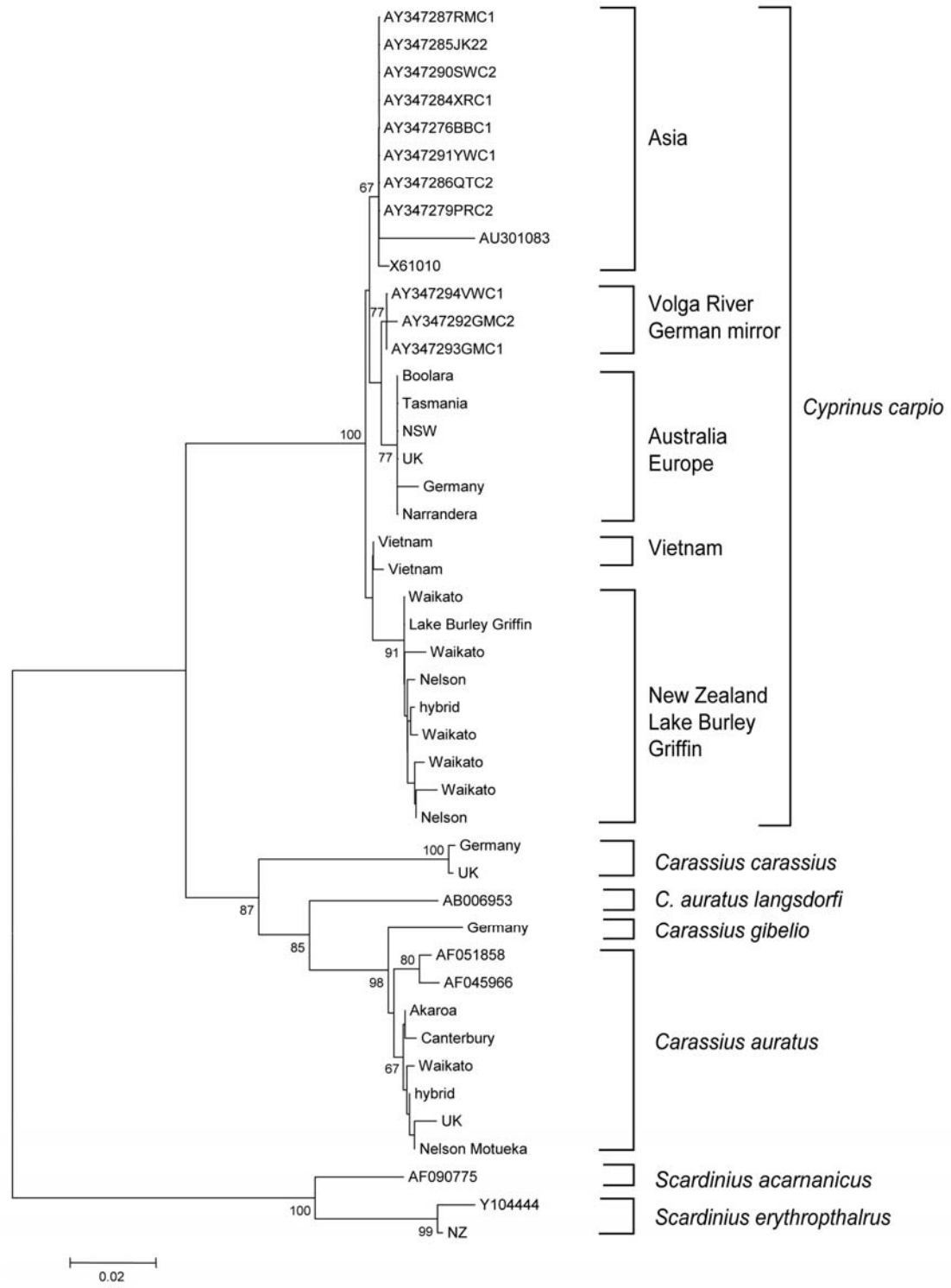

Figure 1B. Phylogenetic relationships of carp from Australia and Vietnam, and carp and goldfish specimens from New Zealand, Germany and the UK based on cytochrome *b* sequences. Scale bars represent an interval of the general time-reversible model (Rodriquez et al. 1990), and numbers at nodes are bootstrap percentages (1000 replicates) based on distance. Trees were rooted with the outgroup rudd *Scardinius erythropthalmus* from New Zealand and GenBank and *Scardinius acarnanicus* from GenBank. Control samples are goldfish *Carassius gibelio* and *C. auratus* from Germany and the UK, respectively; carp *Cyprinus carpio*: from Australia, Germany, UK, and Vietnam; and Crucian carp *Carassius carassius* from Germany and the UK. Code numbers refer to GenBank Accession numbers (and where possible the geographic origin or strain of the carp specimen) and include carp from eastern Europe and Asia (locations unspecified) and the domesticated German mirror carp.

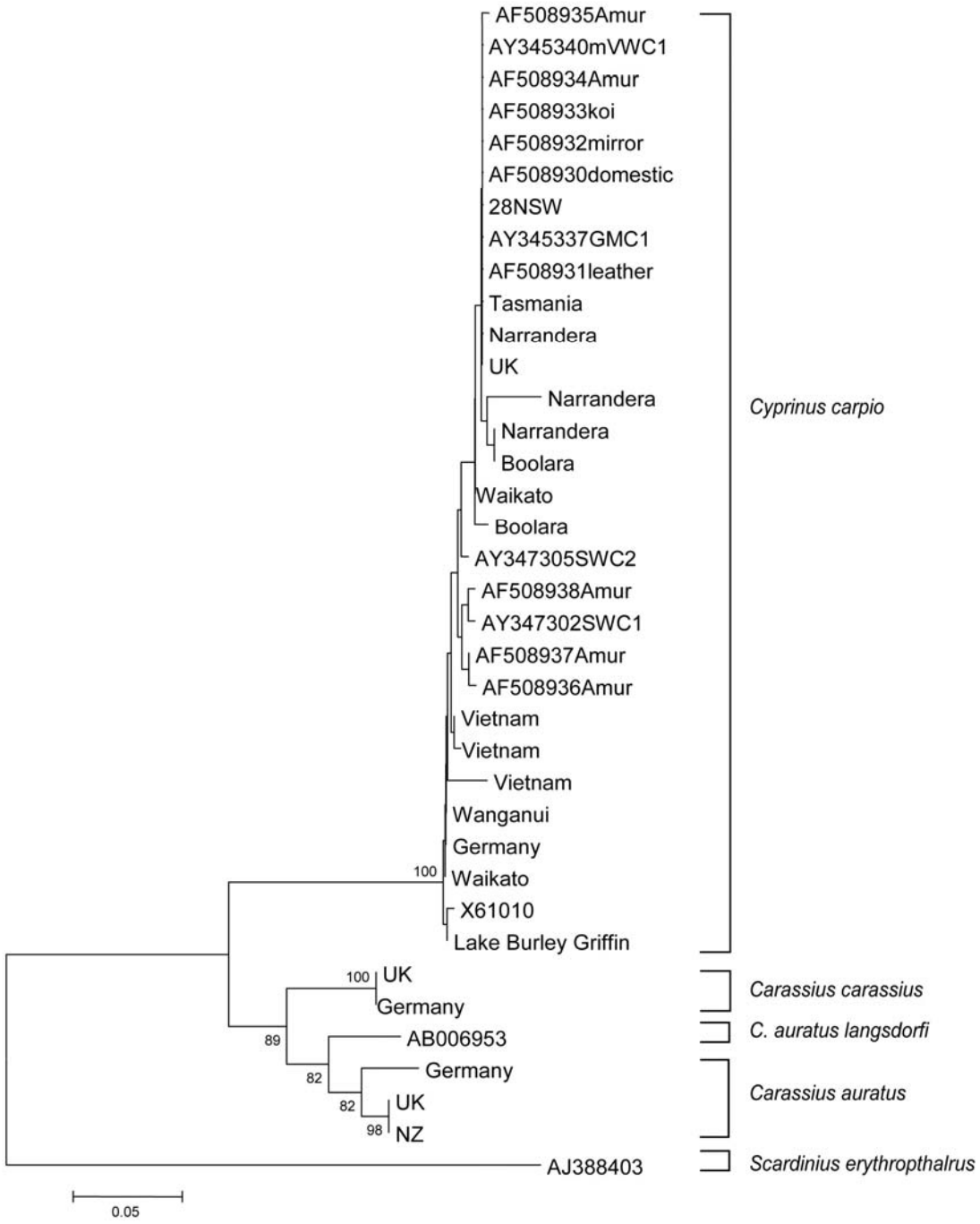

Figure 2. Phylogenetic relationships of carp from Australia, and carp and goldfish specimens from New Zealand, Germany, and the UK, based on control region sequences. Scale bars represent an interval of Tamura & Nei (1993) genetic distance, and numbers at nodes are bootstrap percentages (1000 replicates) based on distance. Trees were rooted with the outgroup rudd *Scardinius erythropthalmus* from New Zealand. Control samples are goldfish *Carassius auratus* from the UK; carp *Cyprinus carpio* from Australia, Germany, UK, and Vietnam; and Crucian carp *Carassius carassius* from Germany and the UK. Code numbers refer to GenBank Accession numbers (and where possible the geographic origin or strain of the carp specimen).

In Australia, an early morphometric and allozyme study reported hybrids between the Yanco strain of carp (red to orange in colour, probably derived from Asia) and goldfish in New South Wales, but not between the Boolara strain of carp (silver grey in colour, probably derived from Europe) and goldfish (Shearer & Mulley 1978). Hume et al. (1983), using meristic and morphometric characters, reported hybrids between the Boolara strain of carp and goldfish; and noted that they had always found hybrids at sites where carp and goldfish were present together. Carp–goldfish hybrids have also been reported in Europe and North America.

Allozymes provide a simple and fast tool for the identification of hybrid carp–goldfish in New Zealand. In future, molecular identification, based on frozen muscle tissue samples, would be sufficient to identify hybrids. Alternatively, hybrids could be identified from ethanol-fixed material, using microsatellite DNA markers.

4.2 CARP GENETIC MARKERS

Several molecular studies of carp have been undertaken over the past decade, with an emphasis on determining the genetic relationships among the wild stocks in Europe and Asia, and the origin of the domestic strains in Europe. Carp were first domesticated ~6000 years BP in China and introduced into Europe during the Roman period. Several subspecies of carp have been described, based on morphological differences, and there is debate over the origin of the European carp, thought to be from either indigenous stocks in the Danube or Asian stocks. Recent carp molecular studies are summarised here as background to the interpretation of the results for the New Zealand specimens.

Froufe et al. (2002) reported identical mtDNA control region (565 bp) sequences among Japanese koi carp (n = 4) and wild, feral, and scale-less varieties of common carp (n = 21) from the Danube. However, individuals from the Amur River exhibited several unique haplotypes, which differed from the European haplotype by 1–12 base substitutions (Froufe et al. 2002). One of the Amur haplotypes was basal, supporting an Asian origin of carp, and Froufe et al. (2002) concluded that a single introduction or domestication event led to the phenotypic diversity in European and koi carp. Subsequently Zhou et al. (2003) reported differences among European carp (*C. carpio carpio*) and Asian carp (*C. carpio haematopterus*) with control region (928 bp) sequences, and concluded that two of the major domesticated strains in central and eastern Europe, the German mirror carp and the Russian scattered-scale carp, have been derived from the European and Asian carps, respectively. Allozyme differences have also been reported among Asian and European carp populations (Kohlman & Kersten 1999, Murakaeva et al. 2003), although it was suggested that these differences may have resulted from cultivation practices (Froufe et al. 2002). The control region sequence data from Froufe et al. (2002) and Zhou et al. (2003) were compared with the New Zealand sequences obtained for this report (Fig. 2).

Gross et al. (2002), using RFLP markers in the ND-3/4 and ND-5/6 regions of the mtDNA, found four distinct genetic groups of carp that corresponded to geography and carp phenotype: European, Amur, Vietnamese, and koi. When

we applied the ND-3/4 markers to carp specimens from New Zealand, none was of the European strain.

Another review of genetic variability in carp populations throughout their distribution range, using allozyme, microsatellite DNA, and mitochondrial DNA markers, reported two distinct groups of carp: Europe/Central Asia and East/South-East Asia, with all three marker systems (Kohlmann et al. 2003). The mean sequence divergence between these groups corresponded to a divergence time of ~500 000 years before present, validating the subspecies status of *C. c. carpio* in Europe and *C. c. haematopterus* in East/South-East Asia (Kohlmann et al. 2003), but the molecular data provided no justification for a separate subspecies status for the central Asian carp (*C. c. aralensis*).

4.3 CARP STRAINS IN NEW ZEALAND

The New Zealand carp were distinguished from carp from Europe, Australia (except ACT), and Vietnam, and from the Crucian carp, by their cytochrome *b* sequences (Fig. 1A). From these sequence data we infer that there were no European or Vietnamese carp *Cyprinus carpio*, or Crucian carp *Carassius carassius*, in the New Zealand samples. The NSW, Tasmanian, and Victorian samples were similar to the *C. carpio* samples from Europe, and based on this result, the Boolara strain in Australia can be excluded as the source of the carp specimens tested in New Zealand. The addition of unpublished carp cytochrome *b* sequences for carp from Russia and Asia did not allow positive identification of the New Zealand carp (Fig. 1B), but further excluded central Asia and eastern Europe as the source of the New Zealand carp.

Our results do not allow exclusion of a possible introduction of koi carp (the Yanco strain) from Australia. The single specimen from Lake Burley Griffin appeared to be similar to the New Zealand carp samples. This specimen had the typical brown coloration of European carp (Tucker pers. comm.) and might have been a hybrid between a female koi and male European carp. Based on an RFLP analysis of the whole mtDNA, Davis et al. (1999) reported that Lake Burley Griffin carp had the same haplotype as carp taken from a pond used by a koi carp breeder. The RFLP method of detecting genetic variation has been largely surpassed by rapid developments in molecular biology, and we sequenced around 1000 bases in the New Zealand koi and carp control samples, compared with the 27 restriction enzymes screened by Davis et al. (1999), which translates into a maximum of 162 bases. Davis et al. (1999) found three haplotypes in Australian carp; one haplotype was common (observed in 72% fish tested) across a wide area in Victoria and New South Wales, and possibly corresponded to the Boolara strain. The second haplotype was rare (observed in 4% of fish tested), and Davis et al. (1999) suggested that this haplotype corresponded to the Yanco strain (= koi) of Shearer & Mulley (1978), based on geographical location, but gave no information on colour of the specimens. A third haplotype was found in a fish pond of a koi-breeder at Bringelly (Davis et al. 1999). All of the Bringelly fish were haplotype 3, as were the majority of fish from Lake Crescent (Tasmania) and Lake Burley Griffin (ACT) (Davis et al. 1999). It is possible that haplotype 3 is diagnostic for koi; but unfortunately

there is no information on the colour of the specimens to relate to the DNA haplotypes. The Bringelly fish may have been stocked into other sites.

It is unlikely that molecular markers could be used to determine if New Zealand koi were introduced from Australia or Asia, unless one source population had been subject to selective breeding and/or passed through a population bottleneck. Ultimately the koi in both Australia and New Zealand have been derived from Asia.

The mtDNA control region sequences did not allow clear separation of carp specimens from Australia, Germany, Vietnam, the UK and New Zealand, and thus this region of mtDNA does not allow identification of the strain of carp in New Zealand. Froufe et al. (2002) also reported identical mtDNA control region (565 bp) sequences among Japanese koi carp (n = 4) and wild, feral, and scaleless varieties of common carp (n = 21) from the Danube, and some of their sequence data were included in our phylogenetic analyses (Fig. 2: Amur, koi, mirror, leather, and domestic carp: GenBank Accession numbers AF508930–38). Zhou et al. (2003) reported differences among European (*C. carpio carpio*) and Asian (*C. carpio haematopterus*) carp with control region (928 bp) sequences. The corresponding 409 bp sequences were included in the phylogenetic analyses (Fig. 2. GenBank Accession numbers AY347302, AY347305, AY345337, and AY345340), but provide no significant differentiation among the carp samples (Fig. 2).

The mtDNA cytochrome *b* sequence data show that the New Zealand carp are not the European or Vietnamese strain. Lack of control samples from the Amur and koi strains did not allow positive strain identification with the key cytochrome *b* markers, but based on colour alone the New Zealand carp are all koi-like. Control samples of koi carp from Japan are required to confirm the molecular identity of the carp in New Zealand.

4.4 GOLDFISH IN NEW ZEALAND

Based on the mtDNA sequence data for cytocrome *b* and the control region, the New Zealand goldfish are the European goldfish *Carassius auratus* and not the East Asian *Carassius gibelio*.

5. Acknowledgements

Australian samples, most likely of the Boolara strain, were supplied by Dr Michael Lowry (NSW Fisheries, Cronulla, NSW); additional Australian samples from ACT, NSW, Tasmania, and Victoria (= the Boolara strain) were supplied by Brad Tucker (Pest Animal Control Cooperative Research Centre, Canberra). German samples were supplied by Dr Bernd Haenfling (Molecular Ecology and Fisheries Genetics, Dept of Biological Sciences, University of Hull, UK); UK samples by Dan Smallwood (National Fisheries Laboratory, Environment Agency, Brampton, Cambridgeshire, UK); and Vietnamese samples by Prof.

Klaus Kohlmann (Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Germany). We are also grateful to Dr Bernd Haenfling for testing some of our samples with microsatellite markers and allowing us to give his results here.

6. References

Baker, C.S.; Perry, E.; Chambers, G.K.; Smith, P.J. 1995: Population variation in the mitochondrial cytochrome *b* gene of the orange roughy *Hoplostethus atlanticus* and the hoki *Macruronus novaezelandiae*. *Marine Biology 122*: 503–509.

Benson, P.G.; Smith, P.J. 1989: Manual of electrophoretic techniques. *Fisheries Research Technical Report 13*. 32 p.

Chang, Y.-S., Huang, F.-L., Lo, T.-B. 1994: The complete nucleotide sequence and gene organisation of carp (*Cyprinus carpio*) mitochondrial genome. *Journal of Molecular Evolution 38*: 138–155.

Davis, K.M.; Dixon, P.I.; Harris, J.H. 1999: Allozyme and mitochondrial DNA analysis of carp, *Cyprinus carpio* L., from south-eastern Australia. *Marine and Freshwater Research 50*: 253–260.

Felsenstein, J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39*: 783–791.

Froufe, E.; Magyary, I.; Lehoczky, I.; Weiss, S. 2002: mtDNA sequence data supports an Asian ancestry and single introduction of the common carp into the Danube Basin. *Journal of Fish Biology 61*: 301–304.

Gross, R.; Kohlmann, K.; Kersten, P. 2002: PCR-RFLP analysis of the mitochondrial ND-3/4 and ND-5/6 gene polymorphisms in the European and east Asian subspecies of common carp (*Cyprinus carpio* L.). *Aquaculture 204*: 507–516.

Hall, T.A. 1999: Bioedit: a user friendly biological sequence alignment editor and analysis programme for Windows 95/98/NT. *Nucleic Acids Symposium Service 41*: 95–98.

Hume, D.J.; Andrea, R.F.; Morison, A.K. 1983: Interspecific hybridization between carp (*Cyprinus carpio* L.) and goldfish (*Carassius auratus* L.) from Victorian waters. *Australian Journal of Marine & Freshwater Research 34*: 915–919.

Inoue, J.G.; Miya, M.; Aoyama, J.; Ishikawa, S.; Tsukamoto, K; Nishida, M. 2001: Complete mitochondrial DNA sequence of the Japanese eel *Anguilla japonica*. *Fisheries science 67*: 118–125.

Kohlman, K.; Kersten, P. 1999: Genetic variability of German and foreign common carp (*Cyprinus carpio* L.). *Aquaculture 173*: 435–445.

Kohlmann, K.; Gross, R.; Murakaeva, A.; Kersten, P. 2003: Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. *Aquatic Living Resources 16*: 421–431.

Lockhart, J.P.; Steele, M.A.; Hendy, M.D.; Penny, D. 1994: Recovering evolutionary trees under a more realistic model of sequence evolution. *Molecular Biology and Evolution 11*: 605–612.

McCune, A.; Lovejoy, N. 1998: The relative rate of sympatric and allopatric speciation in fishes: tests using DNA sequence divergences between sister species and among clades. Pp. 132–153 in: Hochachka, D.J.; Berlocher, S.H. (Eds): Endless forms: species and speciation. Oxford University Press, New York.

Murakaeva, A.; Kohlmann, K.; Kersten, P.; Kamilov, B.; Khabibullin, D. 2003: Genetic characterization of wild and domesticated common carp (*Cyprinus carpio* L.) populations from Uzbekistan. *Aquaculture 218*: 153–166.

Palumbi, S.; Martin, A.; Romano, S.; McMillan, W.O.; Stice, L.; Grabowski, G. 1991: The simple fool's guide to PCR, version 2.0. University of Hawaii, Honolulu.

Posada, D.; Crandall, K.A. 1998: MODELTEST: testing the model of DNA substitution. *Bioinformatics 14*: 817–818.

Pullan S.; Smith P.J. 1987: Identification of hybrids between koi, *Cyprinus carpio*, and goldfish, *Carassius auratus*. *New Zealand Journal of Marine & Freshwater Research 21*: 41–46.

Rodriquez, F.; Oliver, J.L.; Marin, A.; Medina, J.R. 1990: The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology 142*: 485–501.

Shearer, K.D., Mulley, J.C. 1978: The introduction and distribution of the carp, *Cyprinus carpio* Linnaeus, in Australia. *Australian Journal of Marine & Freshwater Research 29*: 551–563.

Swofford, D.L. 2000: PAUP: phylogenetic analysis using parsimony. Version 4.0b. Smithsonian Institution and Sinauer, Sunderland, Massachusetts.

Taggart, J.B.; Hynes, R.A.; Prodohl, P.A.; Ferguson, A. 1992: A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of Fish Biology 40*: 963–965.

Tamura, K; Nei, M. 1993: Estimating the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution 10*: 512–526.

Wheeler, A. 2000: Status of the crucian carp, *Carassius carassius* (L.), in the UK. *Fisheries Management and Ecology* 7: 315–322.

Zelinskij, YuP.; Dmitrenko, Yu.; Pervozvanskiy, V. 1992: Evaluation of genome interaction from growth characteristics and karyological and morphological characters in triploid hybrids between wild goldfish and carp reared in Karelia. *Journal of Ichthyology 32*: 65–73.

Zhou, J.; Wu, Q.; Wang, Z.; Ye, Y. 2003a: Phylogenetic study for three subspecies of common carp *Cyprinus carpio*, based on sequence analysis of cytochrome b and control region of mtDNA. Unpublished report, GenBank.

Zhou, J.; Wu, Q.; Zhen, Y.; Tong, J. 2003b: Genetic divergence between *Cyprinus carpio carpio* and *Cyprinus carpio haematopterus* as assessed by mitochondrial DNA analysis with emphasis on origin of European carp. *Genetica 119*: 93–97.