Maccullochella ikei, an Endangered Species of Freshwater Cod (Pisces: Percichthyidae) from the Clarence River System, NSW and *M. peelii mariensis*, a New Subspecies from the Mary River System, Qld

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ABSTRACT. The eastern freshwater cod, *Maccullochella ikei*, from the Clarence River system, NSW, is described and *M. peelii mariensis* is described as a new subspecies from the Mary River system, Qld. The Murray cod, *M. p. peelii* and the trout cod, *M. macquariensis*, are indigenous to the Murray-Darling River system. Using electrophoretic data for 19 presumed loci, the coefficient of genetic identity between *M. p. peelii* and *M. ikei* was 0.72 and the banding-patterns of the muscle general protein, liver esterases and five other enzymes, were species-specific. A high proportion of abnormal and inviable hybrid larvae in a cross-breeding experiment indicate that post-zygotic isolating mechanisms have evolved between *M. p. peelii* and *M. ikei*. The coefficient of genetic identity between *M. p. peelii* and *M. p. mariensis* was 0.85 and banding patterns of muscle general protein were diagnostic. Canonical variates analysis using 18 morphological and nine meristic characters, clearly separated both species and the subspecies. *Maccullochella ikei* is distinguished from *M. p. peelii* by having longer pelvic fins, larger orbit length, and larger and morphologically distinct sagittal otoliths. *Maccullochella p. mariensis* differs from *M. p. peelii* by the combination of a deeper and shorter caudal peduncle, longer pelvic fins, larger sagittal otoliths and lesser extension of the first anal pterygiophore towards the first caudal vertebra, and from *M. ikei* by the combination of a deeper caudal peduncle, greater postorbital head length, smaller orbit, larger interorbital width, fewer scale rows below the lateral line, shorter fifth-sixth dorsal spine and lesser extension of the first anal pterygiophore. Although each species has distinctive colouration and mottling, there is some intra-specific variation. *Maccullochella macquariensis* is distinct from the other species by having a straight head profile, jaws of similar length, 14 precaudal vertebrae and distinctive colouration. There was a dramatic reduction in the distribution and abundance of cod in the eastern drainage during the early 1900s and cod were probably extinct in the Richmond and Brisbane River systems by the end of the 1930s. *Maccullochella ikei* and *M. p. mariensis* are each represented by one, small population. Possible causes of the decline, and the management strategies taken to conserve the endangered *M. ikei* are discussed.

The Murray cod *Maccullochella peelii peelii* (Mitchell, 1838) is an Australian native warmwater fish which grows to over 50 kg and is highly regarded because of its size and excellent angling and edible qualities. Murray cod, although no longer common (Rowland, 1989), are found naturally throughout much of the Murray-Darling River system (Fig.1). The trout cod, *M. macquariensis* (Cuvier, 1829) which was once sympatric with *M. p. peelii* in the southern tributaries of the Murray-Darling River system (Berra & Weatherley, 1972), is now found only in the Murray River between Yarrawonga Weir and Tocumwal, and in the upper reaches of Seven Creeks (Ingram *et al.*, 1990) and has been classified as endangered (Harris, 1987).

Murray cod have been introduced into numerous waters outside their natural range including the Cox's, Mulwaree, Nepean and Wollondilly Rivers, Cataract Dam, and several other Sydney water supply dams in the eastern drainage of central NSW (Rowland, 1989). Freshwater cod have also been recorded from the Clarence, Richmond, Brisbane and Mary River systems in the eastern drainage of northern NSW and southern Queensland, but there has always been doubt as to the taxonomic status and origin of the cod in these coastal rivers (Ogilby, 1893, 1895; Anderson, 1916; Lake, 1971). Wilcox (1863) stated that Mr J. Macleay identified a 'Murray River cod' from the Urara
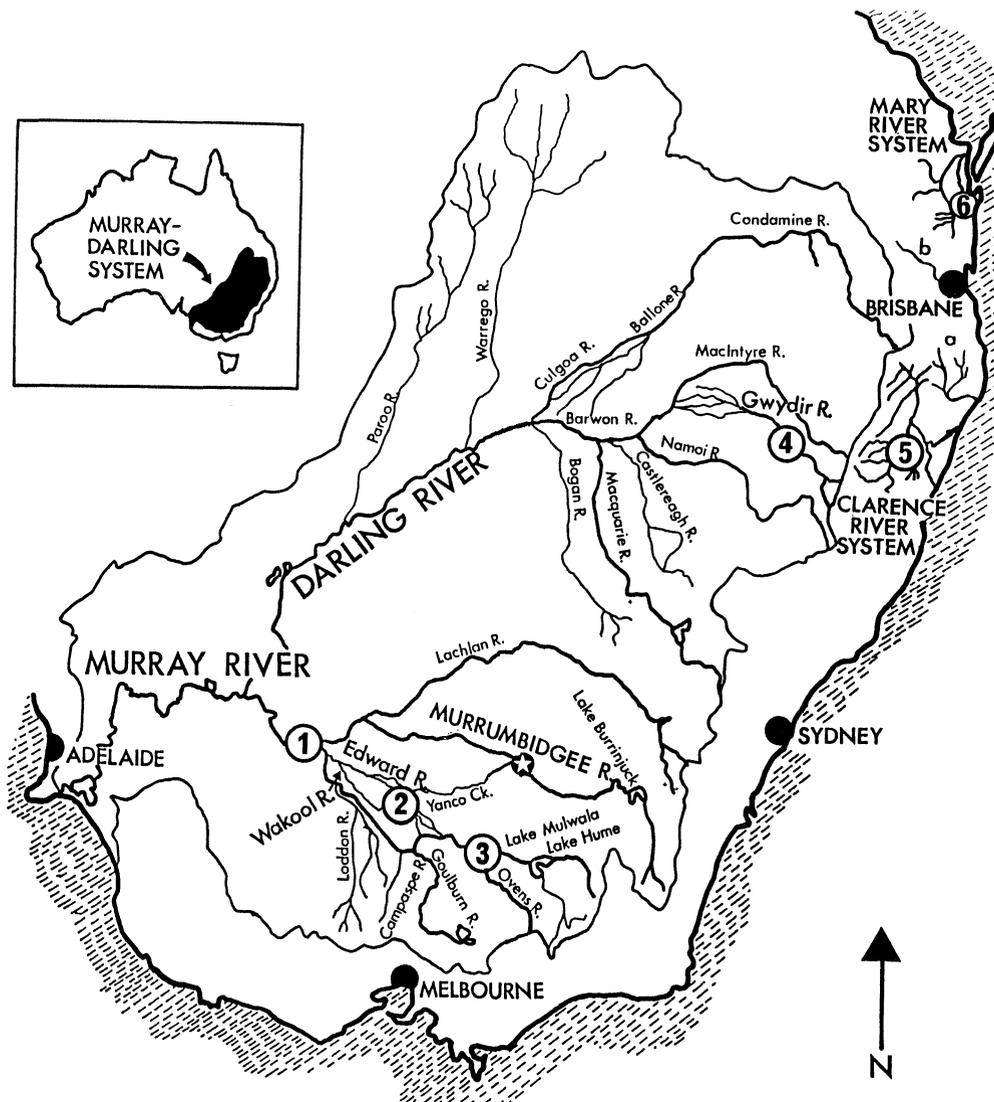
Fig.1. The Murray-Darling, Clarence and Mary River systems: (a) Richmond River system, (b) Brisbane River system. Sampling sites: 1. Murray River; 2. Edward and Wakool Rivers; 3. Lake Mulwala; 4. Gwydir and MacIntyre Rivers; 5. Mann and Nymboida Rivers; 6. Six-mile, Coondoo and Tinana Creeks. Star in circle – Inland Fisheries Research Station.

[sic] (The Orara River is a tributary of the Clarence River) and Hill (1864) stated that Mr James Devlin, who had lived on both the Murrumbidgee and Clarence Rivers, considered that the codfish of the Urara [sic] were identical to those of the western waters. Macleay (1887) obtained a cod from the Richmond River and after comparing it carefully with specimens of *Oligorus macquariensis* and *O. mitchelli* [synonyms of *M. p. peelii* and *M. macquariensis* respectively (Berra & Weatherley, 1972; MacDonald, 1978)] considered that it resembled the latter.

The naturalists on the *Challenger* expedition recorded *O. macquariensis* from the Mary River (Ogilby, 1895), and O'Connor (1897) stated that the Murray cod, *O. macquariensis*, was known to occur in the upper part of the Brisbane River. McCulloch and Whitley (1925) also recorded *O. macquariensis* from these two rivers as well as the tributaries of the Darling River in Queensland. Two forms of cod – 'sharp-nose' and 'boof-headed' – are currently recognised in the Mary system (Merrick & Schmida, 1984), and experienced anglers have long recognised morphological and biological differences between cod from the Mary River and those from the Murray-Darling system in southern Queensland (J. Winkel, personal communication).

Whitley (1937) and Berra & Weatherley (1972) included museum specimens of cod from the Mary River in their systematic reviews of *Maccullochella*, but details of the number and size of these specimens were not given. MacDonald (1978) did not include cod from the eastern drainage in his review of the systematics of Australian freshwater percichthyid fishes. The cod population in the Clarence River system was recognised as a distinct species by Rowland (1985) and a description of *Maccullochella ikei* n.sp., without details of the type material, was published by Rowland (1986). In the current paper, the research to determine the taxonomic status of the cod populations in the eastern drainage is detailed. Data on the type material of *M. ikei* are presented, and *M. peelii mariensis* is described as a new subspecies from the Mary River system. Historical records were examined, and residents of north-eastern NSW and south-eastern Queensland were interviewed to enable a qualitative comparison of the past and present distribution and abundance of cod in the eastern drainage.

Materials and Methods

General. Cod were sampled from the Murray-Darling, Clarence and Mary systems (Fig.1). In addition, Murray cod that had been artificially bred at the Inland Fisheries Research Station (IFRS) and stocked into farm dams near Narrandera, were sampled when one to three years old. The number and range in standard length (SL) of cod sampled from each site are given in Table 1 [Appendix]. After capture, morphometric data were recorded and liver tissue and muscle tissue from midway between the base of the pelvic fin and the anus, were

removed from some specimens, frozen and kept at -20°C until required for electrophoresis. Additional specimens of cod from the Clarence system were examined live in the field, under anaesthetic, and released.

All measurements and counts were made on fresh specimens with the exception of those for the type specimens which were made within two months of fixation in 10% formalin and preservation in 70% alcohol. In the descriptions, proportions and counts for the lectotype of *M. ikei* and the holotype of *M. p. mariensis* are followed, in parentheses, by the range of each character for the paralectotypes, paratypes, other material and other specimens. AMS and QM are prefixes for specimens lodged at the Australian Museum, Sydney and Queensland Museum, Brisbane respectively.

All tables are listed in the Appendix.

Electrophoresis. Electrophoresis was carried out on slab polyacrylamide gels, horizontal starch gels and cellulose acetate sheets using techniques described by Shaw & Prasad (1970), Keenan & Shaklee (1985) and Shaklee & Keenan (1986). Details of the six electrophoretic systems used are given in Table 2 and the proteins that were assayed, the systems and tissues used and the number of cod analysed are given in Table 3. Tissues for starch gel and cellulose acetate electrophoresis were homogenised in a lysing solution (100 mL H_2O, 2 drops MCE, 100 uL NaDP) and centrifuged at 5000 rpm for 5 min at 4°C. Presumptive gene loci are labelled with an italicised abbreviation for the enzyme (Table 3) and multiple loci for a given enzyme are distinguished by numerals, with 1 representing the most anodally migrating isozyme. Allozymes of common mobility are interpreted as if encoded by the same allele. Alleles were designated by their relative mobility, with the most anodal being 100.

The genetic identity and genetic distance between cod from the Murray-Darling, Clarence and Mary systems were estimated using the coefficient of genetic identity (I) and genetic distance (D = -ln I) as defined by Nei (1972). Values of I can range from 0 where the populations being compared share no alleles, to 1, where all alleles are common and have the same frequency. Estimates of the divergence times between cod in the different systems were calculated using t = 5 x 10^6D (Nei, 1975) where t = time since divergence in years, and D = genetic distance.

Morphometrics. Meristics and morphological measurements follow Hubbs & Lagler (1974) except where stated. The respective measurements were made on the left side of the fish where possible using a measuring board (to the nearest 1 mm) and vernier callipers (to nearest 0.1 mm). The post-orbital and orbit lengths, the inter-orbital width and the width between the posterior nares were least-fleshy measurements. Body depth I is the distance between the base of the first dorsal spine and the base of the pelvic fin and body depth II is the maximum depth when the fish is held

vertically by the lower jaw. The length of the pelvic fin was measured from the base of the fin to the tip of the longest filament when extended.

Data on the 27 characters listed in Tables 6 and 7, plus the number of spines in the anal fin, the number of rays in the pelvic fin and the number of pyloric caecae were recorded. Head characters were expressed as a percentage of head length (HL) and all other measurements, including HL, were expressed as a percentage of SL for each specimen. These data were subjected to a canonical variates analysis (CVA; Reyment et al., 1984). Only cod with values for all characters listed in Tables 6 and 7 except body depth I were included in the analysis. The first and second canonical variates were plotted to identify the distinct forms of cod, and the canonical structures were listed to identify the main characters contributing to the variation. The means of each morphometric character for cod from the Clarence system, the Mary system and Murray cod from rivers and farm dams were then compared using Student's t-tests; this analysis was also used to compare the means of the characters for Murray cod from rivers and farm dams and from Lake Mulwala. To reduce the effect of allometric growth of the various body parts on the analysis, only Murray cod (with the exception of those sampled from Lake Mulwala) that were within the length range of cod sampled from the Clarence and Mary systems were analysed. Berra & Weatherley (1972) found that eye diameter and the length of the longest dorsal spine grew allometrically in Murray cod, and so the means of all characters except orbit length and the length of the fifth or sixth dorsal spine were compared between populations of Murray cod.

Osteology. X-rays were made of preserved specimens of Murray cod (n=3), trout cod (3), and cod from the Clarence (4) and Mary (5) systems. The predorsal bones, and the precaudal and caudal vertebrae were counted in each specimen. In this study, vertebrae anterior to the vertebra associated with the first anal pterygiophore were classified as precaudal vertebrae. The length of the first anal pterygiophore was measured (on the X-rays) and expressed as a percentage of the distance from the base of this pterygiophore to the ventral surface of the first caudal vertebra.

Otoliths. The lengths and widths of otoliths from five Murray cod (total length, TL 420-625 mm), five cod from the Clarence system (TL 330-489 mm) and four cod from the Mary system (TL 420-610 mm) were measured using vernier callipers and expressed as a percentage of the TL for each fish. The otoliths were then lightly smeared with graphite to emphasise external morphological features and compared.

Cross-breeding experiment. Four cod (1 male, 3 females) were captured from the Clarence system and transported live to the IFRS for use in a cross-breeding experiment with Murray cod that had originally been

captured from the Murrumbidgee River. All cod were held in earthen ponds until October. Experiments involving cod from the Mary River system and trout cod were not conducted because of the logistical problems with catching, transporting and maintaining the broodfish, the limited facilities at IFRS and the general difficulty of breeding freshwater cod.

Techniques for the handling, injection of human chorionic gonadotrophin (HCG), stripping, and the fertilisation and incubation of eggs are described by Rowland (1988a). The only male cod from the Clarence system died after the injection of HCG and so reciprocal crosses involving this male were not possible. Milt from two Murray cod males were used to fertilise eggs. The eggs from each female were incubated in separate troughs at temperatures ranging from 20 to 24°C. The hatch rate of eggs from each female was determined by expressing the number of larvae that hatched as a percentage of the number of eggs incubated. Larvae from each batch of eggs were sampled daily and examined microscopically, and differences between Murray cod and hybrid larvae were noted.

Results

Electrophoresis – general proteins. The phenotypes of general protein following polyacrylamide gel electrophoresis of cod muscle tissue are shown in Figure 2. The banding patterns at three zones (1, 2, 3) were diagnostic for cod from each of the three systems; cod from the Clarence and Mary systems differed at zone 1, cod from the Clarence and Murray cod at zones 2 and 3, and cod from the Mary system differed from Murray cod at all three zones. The banding patterns of Murray cod from all sampling sites (including Lake Mulwala; No. 24) were indistinguishable, with the exception of fish No. 1 which had a distinct band anodal to the diagnostic fast band at zone 3 (Fig.2). This cod was from the Gwydir River in northern NSW Fish No. 4 was also from this river and its banding pattern was identical to that of all other specimens of Murray cod.

Enzymatic proteins. The ten loci encoding the first eight enzymes listed in Table 3 were monomorphic and fixed for the same allele in all cod. The most anodal isozyme of aspartate amino-transferase (Aat-1) consisted of two bands and the isozymes of the other non-diagnostic enzymes consisted of single, generally intensely-staining bands. The malate dehydrogenase isozyme consisted of two bands, with the most anodal band having greater intensity in tissue from Murray cod and cod from the Mary system, whereas the cathodal band stained more intensely in tissue of cod from the Clarence system. Allelic frequencies at loci of diagnostic enzymes after starch gel and cellulose acetate electrophoresis of cod muscle and liver tissue are given in Table 4.

Mannose-6-phosphate isomerase (MPI). The enzyme MPI varies genetically in a wide variety of organisms but generally behaves as a monomer exhibiting single-banded homozygotes (Shaklee, 1983). All cod examined had a single, intensely-staining band at one locus, *Mpi-1*. Murray cod and cod from the Clarence system were fixed for alternative alleles (100, 87) at this locus, and cod from the Mary system were fixed for the allele characteristic of Murray cod.

Isocitrate dehydrogenase (IDH). The NADP$^+$-dependent IDH of teleost fishes often appears in both supernatant and mitochondrial forms (Engel *et al.*, 1971; Wheat *et al.*, 1973). Two isozymes of IDH were observed after electrophoresis of cod muscle tissue and the lack of interlocus heterodimers suggests that the observed phenotypes represent one supernatant and one mitochondrial loci. All cod were fixed for the same allele at *Idh-1*, but Murray cod and cod from the Clarence system were fixed for alternative alleles at *Idh-2*. The phenotypes at this locus of cod from the Mary system were not determined.

L-iditol dehydrogenase (IDDH). Two loci control the expression of IDDH phenotypes in cod liver tissue. At *Iddh-1*, Murray cod and cod from the Clarence system were fixed for alternative alleles (100, 88). Five cod from the Mary system displayed the allele characteristic of Clarence fish but one cod (captured from Tinana Creek) displayed the allele characteristic of Murray cod. No heterozygous forms were observed and the cod from Tinana Creek was otherwise electrophoretically identical to the other cod from the Mary system.

Glucose-6-phosphate isomerase (GPI). GPI in fishes is a dimeric molecule and multiple isozymes result from the interaction of subunits (Avise & Kitto, 1973). Two types of subunits predominate in nearly all tissues (Shaklee *et al.*, 1974). The GPI phenotypes observed after electrophoresis of cod muscle tissue were presumed to represent the homodimers *Gpi-A$_2$* and *Gpi-B$_2$* and the *AB* heterodimer. *Gpi-B$_2$* did not stain intensely although slight variation was evident, different phenotypes were not allocated to individual fish. *Gpi-A$_2$* stained intensely. Murray cod and cod from the Mary system were fixed for the 'fast' band and cod from the Clarence were fixed for the 'slow' band.

Glycerol-3-phosphate dehydrogenase (G3PDH). Broad bands of G3PDH activity were apparent after starch gel electrophoresis of cod muscle tissue. There were two relative mobilities of the bands – 'fast' and 'slow' – and it was assumed that these represented alleles at a single G3PDH locus. Again, Murray cod and cod from the Clarence system were fixed for alternative alleles (100, 90) and the cod from the Mary system all had the allele characteristic of Murray cod.

Glucose-6-phosphate dehydrogenase (G6PDH). Multiple allozymes were resolved by electrophoresis of cod liver tissue, but positive interpretation was not possible because the molecular and genetic bases of teleost G6PDH are poorly understood (Shaklee *et al.*, 1974). It is tentatively proposed that two loci control the expression of G6PDH phenotypes in cod liver tissue and that there are five alleles at the *G6pdh-1* locus. Other possible interpretations of the observed phenotypes are
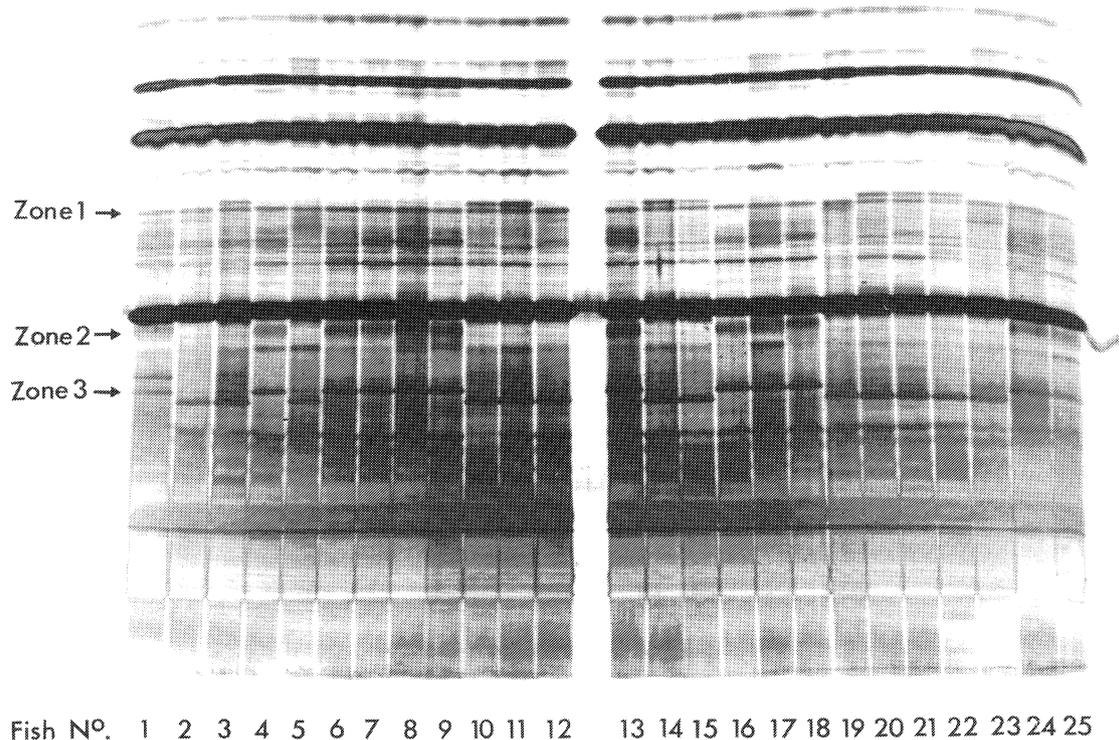
Fig.2. General protein phenotypes of cod after polyacrylamide gel electrophoresis of muscle tissue. *Maccullochella p. peelii*: fish nos 1, 4, 6, 7, 8, 9, 13, 16, 17, 18, 24, 25; *Maccullochella ikei*: fish nos 2, 5, 12, 19, 23; *Maccullochella p. mariensis*: fish nos 3, 10, 11, 14, 15, 20, 21, 22.

multiple loci, heterodimers and post-translation changes. There was a significant difference ($X^2=183.56$, df=8, P less than 0.005) in the frequency of the presumed alleles at *G6pdh-1* between Murray cod and cod from the Clarence and Mary systems. The two alleles (92, 69) with the highest frequency in Murray cod, were either absent or present at low frequencies in cod from the eastern drainage (Table 4). Although there appeared to be some variation at *G6pdh-2*, resolution of this locus was relatively poor and so different phenotypes were not allocated to individual fish.

Esterases (EST). There were three major zones of activity in cod liver tissue and at zone 2, bands of different mobility characterised Murray cod and cod from the Clarence system. EST phenotypes of cod from the Mary system were not determined because of poor resolution.

Genetic identity, genetic distance and time since divergence. Electrophoretic data from 19 loci were used

to calculate the coefficients of genetic identity. The data for general protein, esterases, malate dehydrogenase (NADP⁺) and *G6pdh-2* were not used because the genetic basis of the former two proteins is not known and the interpretation of phenotypes at *Mdh (NADP⁺)-1* and *G6pdh-2* was uncertain.

The coefficients of genetic identity (I) and the genetic distance (D) between Murray cod, cod from the Clarence and Mary systems and trout cod [data from MacDonald (1978)] are given in Table 5.

The electrophoretic data derived from the enzymatic proteins strongly suggest that cod in the Clarence system represent a separate species to *M. p. peelii* (I=0.72, D=0.33) and that the population is not conspecific with cod in the Mary system (I=0.73, D=0.32). The electrophoretic data also suggest that the cod population in the Mary system is more closely related to *M. p. peelii* (I=0.85, D=0.16) than to cod in the Clarence system.

The estimates of the time of divergence of *M. p. peelii* and cod in the Clarence and Mary systems from a
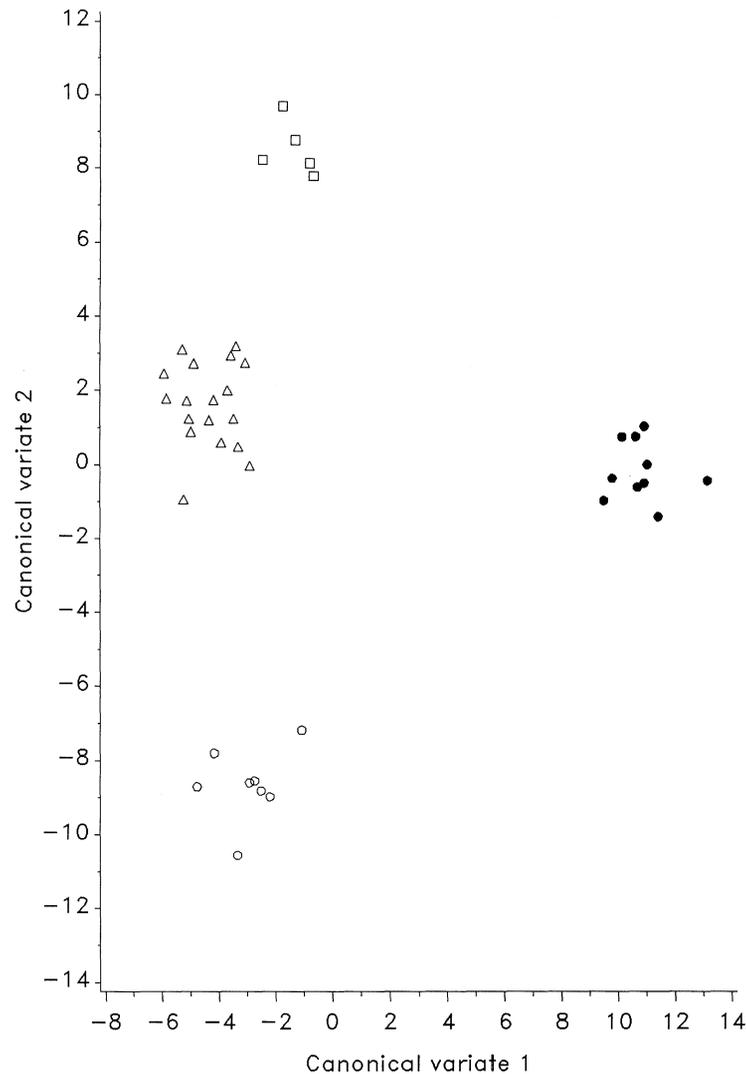
Fig.3. Plot of first and second canonical variates. Dot – *M. ikei*, circle – *M. p. mariensis*, square – *M. p. peelii* ex Lake Mulwala, triangle – *M. p. peelii* ex rivers and farm dams.

common ancestor are 1.7 and 0.8 million years ago (mya) respectively.

Morphometrics – canonical variates analysis. The number of spines in the anal fin (3), rays in the pelvic fin (5) and pyloric caecae (4) were the same in all cod examined and hence not entered into the CVA. The CVA of the remaining 27 characters separated cod into four distinct groups: cod from the Clarence system; cod from the Mary system; Murray cod from rivers and farm dams; Murray cod from Lake Mulwala (Fig.3). Cod from the Clarence system were separated from the other populations by the first canonical variate on the basis of postorbital head length, scale rows below the lateral line, length of the fifth/sixth dorsal spine, length of the pelvic fin and interorbital width. The second canonical variate separated the remaining three populations on the basis of length of pelvic fin, anal base, depth of caudal peduncle, and orbit length.

The means of seven morphometric characters differed significantly (P less than 0.01) between Murray cod from the rivers (and farm dams) and those sampled from Lake Mulwala (Table 6). These data demonstrate substantial intraspecific morphological variation between populations in rivers and small, impounded waters and those in large, impounded waters.

Comparison of Murray cod and cod from the Clarence system. These cod have distinctive colouration. Cod from the Clarence system are generally yellow-green to golden with black to very dark green, heavily reticulated mottling on the dorsal surface, head and sides, whereas in Murray cod the colour ranges from cream to olive to yellow-green with relatively smaller, pale to dark green mottling often with small dark spots between the reticulation (Fig.4). The pelvic fin of Clarence cod is clear, whereas in Murray cod this fin is usually whitish.

The means of 13 morphometric characters differed significantly (P less than 0.01) between Murray cod and cod from the Clarence system (Table 7). The ranges of the length of the pelvic fin (expressed as a percentage of SL) do not overlap, thus providing a diagnostic character. The second filament on the first ray of the pelvic fin is usually extended in cod from the Clarence system compared to Murray cod (Fig.4). The fine tip of this filament may be damaged or missing in preserved specimens. Orbit length is also diagnostic.

Cod from the Mary system. These cod differ significantly (P less than 0.01) in ten characters from Clarence cod and in five characters from Murray cod (Table 7). Although there is no single morphometric character which is completely diagnostic because the ranges of all characters overlap, a combination of characters enables diagnosis. These data and the results of the CVA indicate that cod from the Mary system are morphometrically more similar to Murray cod. However, the means of the length of the pelvic fin (expressed as a percentage of SL) were significantly

different (P less than 0.001), and the range of this character in the Mary system population lay mainly within the range of the character for the Clarence population (Table 7). The relatively long pelvic fin is therefore common to the populations of cod in the eastern drainage. Cod in the Mary system also have a dark, heavily reticulated mottled pattern, similar to cod in the Clarence system (Fig.4).

Osteology

Cod from the Clarence system and Murray cod had a total of 35 vertebrae, consisting of 15 precaudal and 20 caudal vertebrae. The three trout cod examined each had 14 precaudal and 21 caudal vertebrae and three of the five cod from the Mary system had 35 (15 + 20) vertebrae, while the other two had only 34 (15 + 19) vertebrae (Table 8). The extension of the first anal pterygiophore towards the first caudal vertebra is greater in Murray cod and cod from the Clarence system than in trout cod and cod from the Mary system.

Otoliths

The sagittal otoliths of *Maccullochella* spp. are relatively large and long (length:width, 2.2-2.8:1), and show some inter-specific differences. Otoliths of cod from the Clarence system (otolith length 3.2-3.5% of fish TL) and the Mary system (2.9-3.3% TL) are larger than otoliths from similar-sized Murray cod (2.5-2.9% TL). In cod from the Clarence system the anterior edge of the rostrum of each otolith is more rounded and has a distinctly smaller antirostrum than otoliths in Murray cod. The medial dorsal surface is slightly indented whereas in Murray cod this surface is slightly convex. The morphology of otoliths in cod from the Mary system appears intermediate to those in Murray cod and cod from the Clarence system.

Cross-breeding Experiment

The results of the hormone-induced breeding trials are presented in Table 9. Dosages of 500 and 1000 IU/kg HCG did not induce ovulation in cod from the Clarence system, however, as expected (Rowland, 1988a) ovulation had commenced in female Murray cod 48 h after the injection of 1000 IU/kg HCG. Two batches of eggs were stripped after different latent periods from each female. The hatch rates of eggs stripped 48 and 48 h after injection were 3 and 58% respectively, and the hatch rates of eggs stripped 49 and 50 h after injection were 90 and 75%. Approximately 96,200 eggs were stripped and a total of 50,125 larvae hatched. All larvae underwent normal development during yolk sac

Fig.4. (top) *Maccullochella peelii peelii* (female, 495 mm SL, 554 mm TL, 3.1 kg total weight, left operculum damaged), (upper middle) *Maccullochella ikei* (female, 515 mm SL, 610 mm TL, 3.4 kg total weight) (photographed live, under anaesthetic by Ken Bock), (lower middle) *Maccullochella peelii mariensis* n.subsp. (photograph, Gunther Schmida) (bottom) *Maccullochella macquariensis* (250 mm TL) (photograph, Gunther Schmida).

absorption, confirming that the milt from the two males was viable.

Ovulation had commenced in a female cod from the Clarence system 46 h after the injection of 2000 IU/kg HCG. Approximately 6,750 eggs were stripped and fertilised using milt from the two Murray cod males. An additional 1,900 eggs were stripped and fertilised two hours later. Hatching commenced 120 h after fertilisation. A total of 777 eggs hatched (9% hatch rate), comprising 147 (2%) of those stripped 46 h after injection and 630 (33%) stripped after 48 h (Table 9).

Most hybrid larvae that hatched from both batches of eggs were obviously deformed. The deformities ranged from gross malformations of the head, body and tail (Fig.5a) to scoliosis and/or lordosis (Fig.5b). Other abnormalities observed in most hybrid larvae were: malformation of the fins, in particular the caudal and pectoral fins; the lack of, or decreased size of the oil droplet; a relatively pale pink colour of blood in comparison to the bright red blood of Murray cod larvae. Abnormal larvae of Murray cod are very uncommon even when hatch rates are low due to

suboptimal induced-spawning conditions (Rowland, 1988a).

There were also some developmental differences between Murray cod and the small number of apparently 'normal' hybrid larvae. The oil droplet in these hybrid larvae was spherical and positioned below the gills, whereas in Murray cod larvae the oil droplet was oval-shaped and situated below the eyes (Fig.6). The cardiac cavity, when viewed laterally, was distinctly narrower in Murray cod than in hybrid larvae.

At the completion of yolk sac absorption, approximately ten days after the completion of hatching, only 226 hybrid larvae were alive (29% of the total that had hatched). At this stage of development, hybrid larvae averaged 10.7 mm TL (n=12) and were significantly smaller (t=10.3, P less than 0.01) than same-aged Murray cod larvae, that averaged 13 mm TL (n=12). Most hybrid larvae were much less active and died within two weeks of yolk sac absorption.

The abnormalities observed in hybrid larvae, the high mortalities during and after yolk sac absorption and the developmental differences between Murray cod and
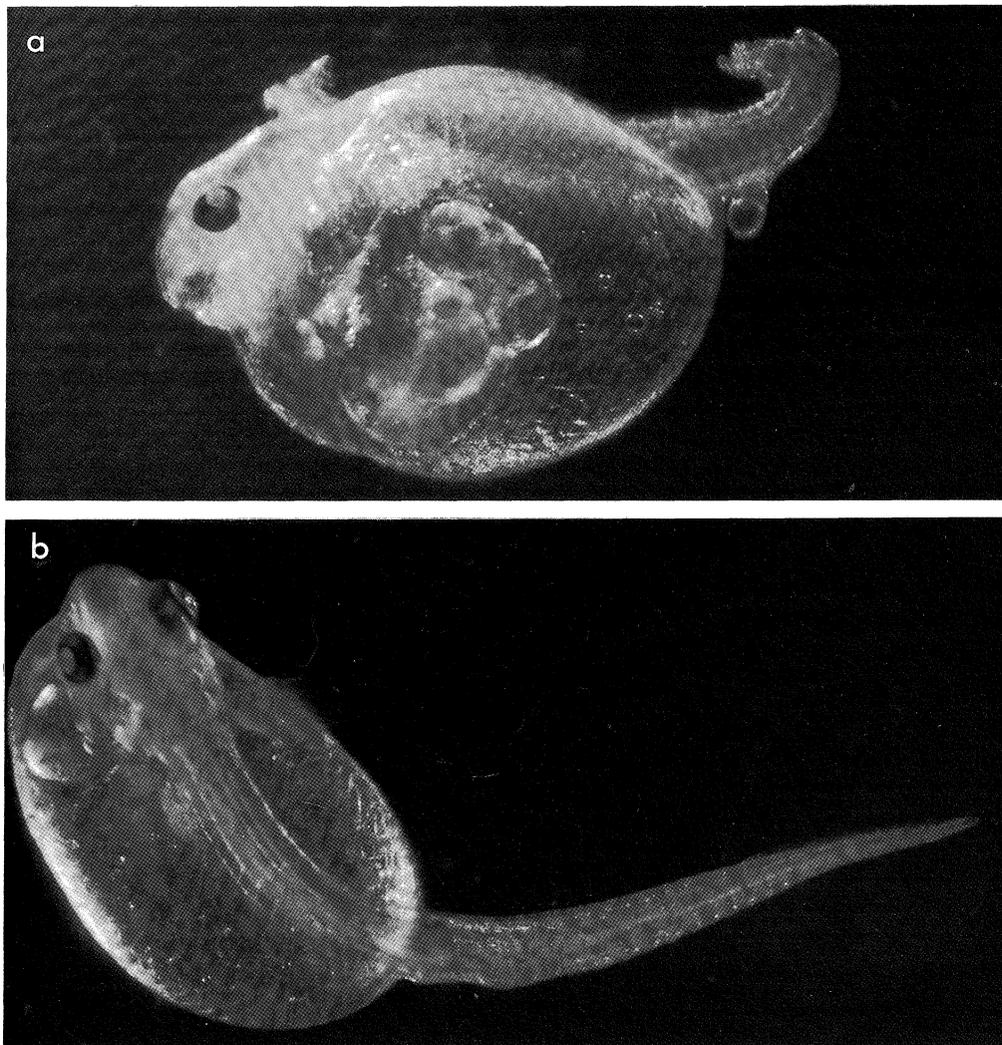
Fig.5. Abnormal larvae (50 h old) from a cross between eastern freshwater cod and Murray cod (x22), (a) grossly abnormal larva, (b) larva with scoliosis (photograph, P.M. Selosse).
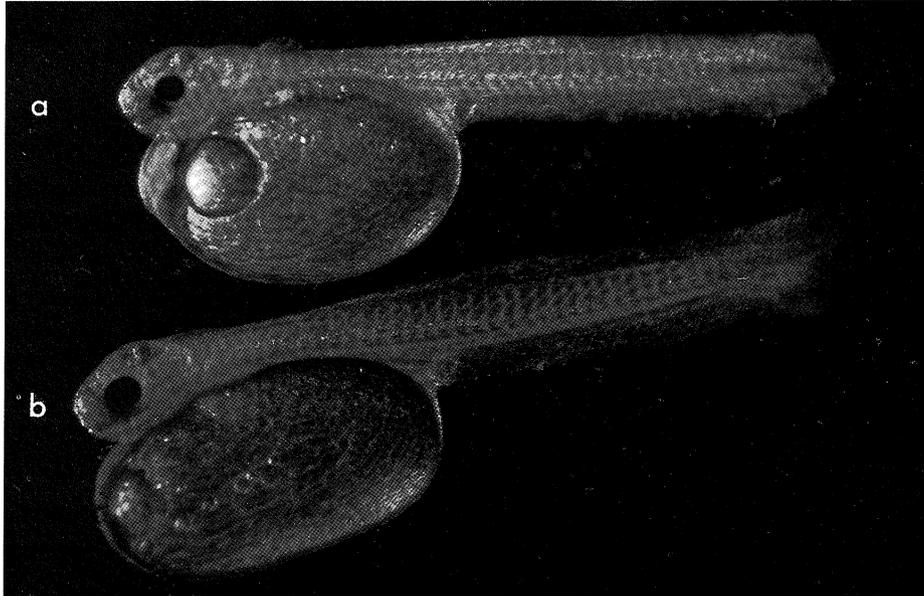
Fig.6. Cod yolk sac larvae 72 h old (x15), (a) from cross between eastern freshwater cod and Murray cod and (b) Murray cod (photograph, P.M. Selosse).

hybrid larvae, suggest that hybrid breakdown was occurring and that post-zygotic isolating mechanisms have evolved between *M. p. peelii* and the cod in the Clarence system.

As a result of the morphological and genetic differences between *M. p. peelii* and cod from the Clarence system, and the inviability of hybrid larvae, the eastern freshwater cod is considered to be a distinct species.

Maccullochella ikei Rowland, 1986

eastern freshwater cod

Fig.7

Gristes peelii.–Bennett, 1864: 81 (in part, in rivers of the eastern ranges).

Macquaria peelii.–Anon., 1859: 5 (large fish from Eurara [sic] River donated to Australian Museum by Mr Wilcox.).–Angas, 1860: 5 (from the Clarence River presented by Mr J.F. Wilcox).

Oligorus macquariensis.–Macleay *et al.*, 1880: 36 (in part, in branches of the Clarence).–Tenison-Woods, 1883: 103 (in part, in a few of the eastern rivers to the north of Sydney).–Ogilby, 1886: 13 (in part, northern rivers of the eastern watershed of NSW).–Ogilby, 1893: 19 (in part, found in some of the northern coastal rivers of the Colony).–Thompson, 1893: 21 (in part, in Clarence system).–Ogilby, 1895: 71, 72 (in part, native of the Richmond and Clarence Rivers district).–McCulloch, 1922: 46, 57 (in part, in the headwaters of some northern streams).–MuCulloch, 1927: 57 (in parts, the headwater of some northern streams).

Oligorus mitchelli.–Macleay, 1887: 981, 982 (in part, specimen from Richmond River).

Maccullochella macquariensis.–Whitley, 1937: 126 (in part, Richmond and Clarence systems).–Lake, 1959: 5 (in part, 2 coastal rivers – the Richmond and Clarence).–Lake, 1967: 31 (in part, in a few coastal streams in northern NSW).–Roughley, 1968: 133 (in part, headwaters of Richmond and Clarence Rivers).–Lake, 1971: 33 (in part, Clarence and Richmond Rivers).–Grant, 1987: 435 (in part, Clarence and Richmond Rivers).

Maccullochella peeli.–Berra & Weatherley, 1972: 60, 61 (in part, Clarence River).–Lake, 1978: 49 (in part, Clarence and Richmond Rivers).–MacDonald, 1978: 668 (in part, Clarence and Richmond Rivers).–Llewellyn & MacDonald, 1980: 143 (in part, Richmond and Clarence Rivers).–Pollard *et al.*, 1980: 246 (in part, Richmond and Clarence Rivers).–Llewellyn, 1983: 15 (in part, north coast rivers, possibly introduced).

Maccullochella sp.–Merrick & Schmida, 1984: 201 (Clarence River).–Harris, 1987: 8, 39 (coastal NSW rivers).–Allen, 1989: 138, 142, 143 (Clarence River system).–Ingram *et al.*, 1990: 179, 180 (Clarence River system).–Pollard *et al.*, 1990: 74 (eastern freshwater cod).

Maccullochella ikei Rowland, 1986: 16,17 (Clarence River system).

Maccullochella ikei.–Rowland, 1985 (unpublished): iv, 23-66 (Clarence River system).–Rowland, 1988b: 18, 19, 20, 22 (Clarence River system).–Rowland, 1988c: 168, 170 (some southern tributaries of the Clarence River system).–Hoser, 1991: 60 (Clarence River system).

Type material. LECTOTYPE, AMS I.31090-001, 410 mm, female, NSW, Nymboida River, at junction with Mann River (29°39'S 152°30'E), granite rocks and gravel to 4 m, angled, K. Clark, 21 Nov. 1984. PARALECTOTYPES, QM I27990, 422 mm, female, Mann River, 2 km upstream of junction with Nymboida River (29°38'S 152°29'E), 1 m, angled, R. Cockbain, 8 June 1982; AMS I.31089-001, 385 mm, female, same location as lectotype, angled, R. Lockwood, 21 Nov. 1984; 503 mm, female, same location as

lectotype, angled, C. Graham, 31 Sept. 1981 (specimen lost).

Other material. AMS I.31090-002, 2: 407-410 mm, males, Mann River, 6 km upstream of Jackadgery (29°37'S 152°33'E), gill net, S. Rowland, 12 Apr. 1986; AMS I.31090-001, 2: 305-320 mm, immature, same location as lectotype, gill net, S. Rowland, 13 Apr. 1986; QM I27991, 395 mm, female, Nymboida River, Ramornie State Forest (29°43'S 152°34'E), 2 m, angled, G. Stewart, 7 Apr. 1987; QM I27992, 355 mm, female, same locality as lectotype, gill net, S. Rowland, 27 Sept. 1987; AMS I.31092-001, 355 mm, female Nymboida River, Buccarumbi (29°51'S 152°35'E), 3 m, angled, F. Prokop, 4 May 1988; AMS I.31093-001, 2: 375-456 mm, males, same location as preceding, 1-3 m, angled, F. Prokop & G. Stewart, 18 May 1988; QM I27993, 440 mm, male, same location as preceding, 2 m, angled, K. Clark, 3 Aug. 1988.

Other specimens. These were examined live in the field and released or disposed of (18: 215-515 mm), as for lectotype, paralectotypes and other material.

Diagnosis. A species of freshwater cod, *Maccullochella*, differing from *M. p. peelii* by having longer pelvic fins, larger orbit length, larger and morphologically distinct sagittal otoliths, and distinctive colouration, from *M. p. mariensis* by the combination of a narrower caudal peduncle, shorter post-orbital head length, larger orbit, shorter inter-orbital width, more scale rows below the lateral line, longer fifth/sixth dorsal spine, and greater extension of the first anal pterygiophore towards the vertebral column, and from *M. macquariensis* by having a concave head profile, protruding lower jaw, 15 precaudal vertebrae, greater extension of the first anal pterygiophore towards the vertebral column and distinctive colouration.

Description. Details of morphometrics of the lectotype, paralectotypes and other material and specimens are given in Table 10.

Dorsal fin XII, 14 (X-XII, 13-16); anal fin III, 12 (III, 11-12); pectoral fin rays 17 (16-19); pelvic fin I, 5; caudal fin rays 18 (17-19); predorsal bones 2 (2-3); precaudal vertebrae 15 (15); caudal vertebrae 20 (20).

Large, elongate, deep-bodied fish; slightly compressed. Depth from dorsal origin to base pelvic fin 28.3 (26.0-

30.3)% SL. Body dorsal profile convex, highest above pectoral base. Caudal peduncle elongated, 19.8 (19.8-24.8)% SL.

Head broad, width 62.1 (46.0-66.2)% HL; moderately compressed; profile distinctly concave. Lower jaw protruding beyond upper; mouth large, gape extending to posterior border of eye. Eyes moderately large, dorsolateral; orbit length 14.3 (12.0-19.0)% HL. Irregular rows of villiform, curved teeth in jaws, on vomer and palatines. Operculum with fleshy margin; 2 spines, lower spine largest. Edge of preoperculum smooth.

Lateral line distinct, following dorsal profile, extending to base of caudal fin; 70 (65-82) scales in lateral line. Body covered by small scales, mostly ctenoid; cheeks and opercular scaled, snout naked.

Pelvic fin inserted in advance of pectoral base; first ray elongated into 2 filaments, second long, tapering to fine tip; pelvic fin 17.9 (16.6-21.0)% SL. Pectoral fin large, 16.7 (14.2-19.0)% SL; upper rays longest. Dorsal fin origin posterior to pectoral and pelvic bases. Dorsal spines strong, fifth-sixth longest 10.8 (8.5-13.0)% SL; soft dorsal fin rounded. Anal fin opposite soft dorsal, rounded; second spine largest. Caudal fin large, rounded.

Colour in life and in alcohol. In life yellow-green to golden with black to very dark green, heavily reticulated mottling on dorsal surface, head and sides, extending onto ventral surface in some specimens. Ventral surface of head and body dark grey; becoming grey-white when in turbid water (Fig.4). Dorsal, pectoral, caudal and anal fins clearish to very dark grey-green with mottling on their bases. Thin pale-grey to whitish margins sometimes present on soft dorsal, anal and caudal fins. Pelvic fin colourless, translucent; filaments white.

In alcohol, grey dorsally with black to dark grey heavily reticulated mottling on dorsal surface, head and sides; ventral surface grey and brownish. Fins dark to grey; pelvic fin white.

Species can attain a large size and cod of 22.7 to 27.3 kg were frequently caught in the Clarence system (Wilcox, 1863); largest specimen reported 41 kg from Koreelah Creek in 1921 (K. Murphy, personal communication).
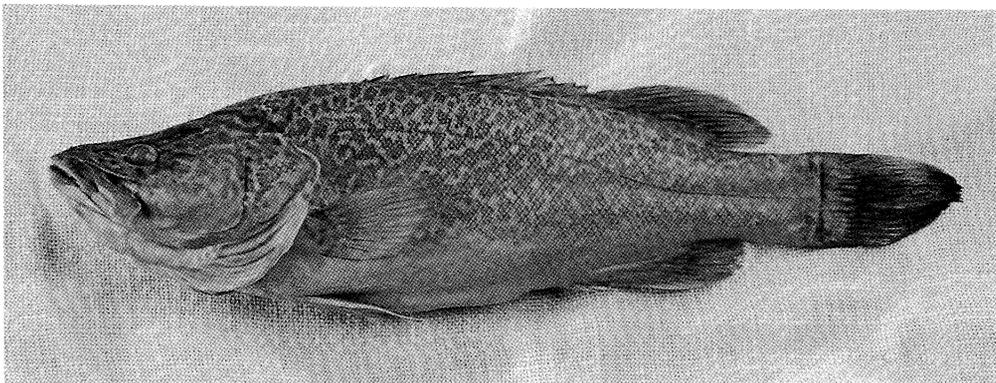

Fig.7. *Maccullochella ikei*. Lectotype (AMS I.31090-001), 410 mm SL (photograph, Kevin Clark).

Etymology. Named *ikei* after the author's late grandfather, Isaac (Ike) Morgan Rowland, who was a great admirer and angler of Australian native inland fishes, in particular Murray cod.

Remarks. No types were listed in the original species description (Rowland, 1986). The type series includes all those specimens from which data were taken as listed in the unpublished thesis of Rowland (1985); the lectotype and paralectotypes are the remaining extant specimens from that syntypic series. Other material includes the specimens collected since 1985.

The population of cod in the Mary system is morphologically and genetically distinct from the other species of *Maccullochella*. The data suggest that this population is more similar to *M. p. peelii* than *M. ikei*, and pending breeding studies and further investigation of the evolutionary relationships within *Maccullochella*, is here described as a subspecies of *M. peelii*.

Maccullochella peelii mariensis n.subsp.

Mary River cod

Fig.4

Oligorus macquariensis.–Ogilby, 1893: 19 (in part, Mary River).–Ogilby, 1895: 71, 72 (in part, Mary River).–McCulloch & Whitley, 1925: 102 (in part, Mary River).

Maccullochella macquariensis.–Whitley, 1937: 128 (in part Mary River).–Roughley, 1968: 133 (in part, Mary River).–Lake, 1971: 33 (in part, Mary River).–Grant, 1982: 785 (in part, Mary and Dawson Rivers).–Grant, 1987: 435 (in part, Mary and Dawson Rivers).

Maccullochella peeli.–Berra & Weatherley, 1972: 60 (in part, Mary River).–Lake, 1978: 49 (in part Mary River).–Llewellyn & MacDonald, 1980: 143 (in part, Mary and Dawson Rivers).

Maccullochella sp.–Merrick & Schmida, 1984: 202 (Mary River system).–Harris, 1987: 9, 39 (Mary River).–Rowland, 1988c: 171 (Mary River system).–Allen, 1989: 142 (Mary River).–Ingram *et al.*, 1990: 179, 180 (Mary River system).–Pollard *et al.*, 1990: 74 (Mary River).

Type material. HOLOTYPE, QM I27994, 382 mm, female, Queensland, Tinana Creek, Bungawatta Station (25°48'S 152°42'E), clay, mud and timber to 3 m, angled, R. Cockbain, 9 Feb. 1984. PARATYPES, QM I27995, 476 mm, female, Coondoo Creek (26°00'S 152°50'E), angled, G. Cook, Dec. 1983; QM I27996, 555 mm, male, Coondoo Creek (26°00'S 152°50'E), gill net, S. Rowland, 7 Feb. 1984; QM I27997, 417 mm, female, same locality as preceding, gill net, S. Rowland, 8 Feb. 1984; QM I27998, 235 mm, immature, Six-mile Creek, 10 km upstream of junction with Mary River

(26°16'S 152°45'E), angled, C. Graham, 8 Feb. 1984; AMS I.32345-001, 440 mm, female, same data as QM I27997; AMS I.32346-001, 387 mm, male, Six-mile Creek, same locality as QM I27998 angled, J. Rowland, 9 Feb. 1984; AMS I.32347-001, 464 mm, female, same locality as preceding, angled, C. Graham, 9 Feb. 1984.

Diagnosis. A subspecies of *Maccullochella peelii* distinguished from the nominal subspecies by the combination of longer pelvic fins, deeper and shorter caudal peduncle, shorter extension of first anal pterygiophore towards vertebral column, larger sagittal otoliths and distinctive colouration, from *M. ikei* by the combination of deeper caudal peduncle, greater postorbital head length, smaller orbit, larger interorbital width, fewer scale rows below lateral line, shorter fifth-sixth dorsal spine and shorter extension of the first anal pterygiophore, and from *M. macquariensis* by having concave head profile, protruding lower jaw, 15 precaudal vertebrae and distinctive colouration.

Description. Details of morphometrics are given in Table 7. Similar general shape to *M. p. peelii* and *M. ikei* but with following details.

Dorsal fin XI, 15; anal fin III, 13 (III, 12-13); pectoral fin rays 18 (19-20); pelvic fin I, 5; caudal fin rays 18 (16-18); predorsal bones 2 (2-3); precaudal vertebrae 15; caudal vertebrae 19 (19-20).

Depth from dorsal origin to base pelvic fin 31.6 (28.3-33.6)% SL. Caudal peduncle elongated 19.3 (19.0-21.4)% SL and relatively deep 13.5 (12.3-14.2)% SL.

Head width 65.0 (47.8-64.6)% HL; orbit length 13.9 (10.0-13.8)% HL.

Lateral line scales 67 (68-74); pelvic fin 18.4 (16.4-19.8)% SL; pectoral fin 18.3 (16.5-19.3)% SL; fifth-sixth dorsal spine longest 9.3 (6.1-9.8)% SL.

Colour in life and alcohol. In life yellowish to pale green with dark heavily reticulated mottling on dorsal surface, head and sides, extending onto ventral surface in some specimens. Ventral surface of head and body grey-green to whitish. Dorsal, pectoral, caudal and anal fins clearish to dark with grey-green mottling on their bases. Thin whitish margins on soft dorsal, anal and caudal fins. Pelvic fin colourless to whitish; filaments white.

In alcohol, grey dorsally and grey-brown on sides with dark reticulated mottling on dorsal surface, head and sides; ventral surface grey-brown. Fins dark to grey-brown; pelvic fin white.

Subspecies can attain a large size and reported to grow to 23.5 kg (Merrick & Schmida, 1984).

Etymology. Named *mariensis* after the Mary River in which the new subspecies is endemic.

Key to the Species of *Maccullochella* in South-eastern Australia

1. Head profile straight. Upper jaw overhangs lower jaw. Blue-grey dorsally with speckled pattern of dark grey to black spots and small bars dorsally and laterally. Precaudal vertebrae 14 .... *Maccullochella macquariensis*

——Head profile concave. Jaws equal or lower jaw protruding. Distinct mottled pattern dorsally and laterally. Precaudal vertebrae 15 2

2. Cream to olive to yellow-green with pale to dark green reticulated mottling dorsally and laterally sometimes with small dark spots between reticulation; ventral surface white to creamy-white. Whitish, relatively short pelvic fin 13.2-16.4% standard length. Orbit length 9.7-12.9% head length. Anal rays 12-15. Otolith with prominent antirostrum; otolith length 2.5-2.9% total length .... *Maccullochella peelii peelii*

——Pale green to yellow-green to golden with black to very dark green, heavily reticulated mottling dorsally and laterally, sometimes extending onto ventral surface; ventral surface dark grey to whitish. Clear, relatively long pelvic fin 16.4-20.7% standard length. Otolith with small antirostrum; otolith length 2.9-3.5% total length 3

3. Depth caudal peduncle 12.3-14.2% standard length. Orbit length 10.0-13.8% head length. Interorbital width 25.1-30.4% head length. Scale rows below lateral line 30-34. Fifth/sixth dorsal spine 6.1-9.8% standard length. Anal rays 12-13. First anal pterygiophore length 79-86% distance from its base to first caudal vertebra. Otolith length 2.9-3.3% total length .... *Maccullochella peelii mariensis*

——Depth caudal peduncle 10.7-12.5% standard length. Orbit length 12.0-19.0% head length. Interorbital width 20.8-27.5% head length. Scale rows below lateral line 32-52. Fifth/sixth dorsal spine 8.5-13.0% standard length. Anal rays 11-12. First anal pterygiophore length 89-97% distance from its base to first caudal vertebra. Otolith length 3.2-3.5% total length .... *Maccullochella ikei*

Discussion

Although the relationship between allopatric populations can be difficult to determine, the technique of electrophoresis enables a quantitative analysis of genetic divergence, and when coupled with morphological techniques, information on taxonomic, and phylogenetic relationships between populations is maximised (Hillis, 1987). The values of the coefficients of genetic identity and genetic distance between *M. p. peelii* and *M. ikei* (I=0.72; D=0.33) and between *M. ikei* and *M. p. mariensis* (0.73; 0.32) are consistent with values previously reported between congeneric species of fishes (Avise, 1974; Ayala, 1975; Shaklee & Tamaru, 1981; Shaklee *et al.*, 1982). The species-specific banding patterns of *Mpi-1, Idh-2, Iddh-1, Gpi-A$_2$* and *G3pdh-1*, liver esterases, and the muscle general protein, as well as the morphometric differences and the abnormalities and inviability of hybrid larvae provide strong evidence of the divergence and evolution of reproductive isolation between cod from the Murray-Darling and Clarence systems.

The 'subspecies' taxon is generally used to arbitrarily identify geographically isolated populations that have undergone some morphological and/or genetic change (Dobzhansky, 1970; Frankel & Soulé, 1981). The cod population in the Mary system is genetically and

morphologically distinct from both *M. p. peelii* and *M. ikei*. Although the phenotypes of muscle general protein (Fig.2) and the character length of pelvic fin suggest a closer relationship to *M. ikei*, the coefficient of genetic identity with *M. peelii* of 0.85 is in the range (0.80-0.96) normally associated with subspecies of fish (Avise, 1974; Avise & Smith, 1977; Philipp *et al.*, 1983; Crabtree & Buth, 1987; Leary *et al.*, 1987). Overall, the genetic and morphometric data suggest a closer relationship to *M. p. peelii*, hence the description of cod in the Mary system as *M. p. mariensis*. The two distinct forms of cod in this system that are recognised by fishermen (Merrick & Schmida, 1984) were not identified in the current study.

It is hypothesised that the cod of the western drainage was the ancestral stock from which founders of *M. ikei* and *M. p. mariensis* become geographically isolated. Fossil Murray cod of Miocene Age (26-7 million years ago [mya]) have been found in the Warrumbungle Mountains and Cooma regions (Hills, 1946; Taylor *et al.*, 1980). Diatom flora associated with some of these fossils is of the type found with lower Tertiary basalts (Gill, 1970) and so ancestral *Maccullochella* may be up to 60-65 m y old (Rowland, 1989).

The genetic distances and the estimated times of divergence (1.7-0.8 mya) suggest that the vertical uplift associated with the formation of the Great Dividing Range (GDR) was not responsible for the geographic isolation of cod in the eastern drainage. Most of the earth movements which formed the Eastern Highlands took place before the Miocene (Ollier, 1978) and the GDR assumed its present gross character early in the Cainozoic Era which commenced 65 mya (Jones & Veevers, 1980). The westward migration of the dividing range has resulted in complex, large-scale stream capture and short, steep coastal rivers have been successively capturing the headwaters of westerly-flowing streams (Herbert, 1980). The founder populations of *M. ikei* and *M. p. mariensis* could have become isolated in the eastern drainage, during this process of stream capture, as was first suggested by McCulloch (1914) to explain the presence of 'Murray cod' in north-eastern NSW and south-eastern Queensland. Speciation can be very rapid in small founder populations (Mayr, 1982) and the vastly different types of habitats in the coastal river systems compared to the western drainage could have contributed to rapid divergence and speciation. An alternative hypothesis is that cod have made separate invasions of Australian freshwaters from marine environments east and west of the GDR, as has been suggested for some atherinids by Crowley (1990).

Although conspecific populations have been recorded east and west of the GDR (Crowley, 1990) in other species of fish, two previous studies have also identified separate species. Crowley *et al.* (1986) found that the rainbowfish of the coastal systems of northern NSW and southern Queensland is *Melanotaenia duboulayi*, whereas *M. fluviatilis* is confined to the Murray-Darling system, and Dufty (1986) suggested that eastern and western populations of Macquarie perch

(*Macquaria australasia*) are separate species. The findings of these, plus the current study suggest that the GDR has played a role in the speciation of some freshwater fishes in eastern Australia. There is clearly a need to maintain the integrity of native fish faunas within drainages, particularly where there is doubt about the taxonomic and reproductive relationships between populations.

Past and present distribution and abundance of cod in the eastern drainage. Historical reports of the 1800s and early 1900s indicate that large populations of cod were once present in each of the Clarence, Richmond, Brisbane and Mary systems. When the Clarence Valley was first settled in the 1830s, cod were plentiful in all the freshwater reaches of the Clarence River and its tributaries (Bawden, 1888) and fish up to 27.3 kg were frequently caught in the Clarence and Orara Rivers (Wilcox, 1863). Many of the very early settlers "lived off the land" and caught cod and other fish and native animals for food (Wilkinson, 1981). In a letter dated Grafton, 10 May 1866, the distinguished naturalist John MacGillivray wrote of catching 25 cod up to 22.7 kg during a trip to the Wymboida [sic] River (Beaton, 1868). Early settlers who were mainly cedar cutters, found that cod up to 27.3 kg were plentiful in the Richmond River and many of its tributaries (Bundock, 1898; S. King, R. Russ, personal communication). In 1824, the explorers John Oxley and Allan Cunningham recognised a fish that had been caught from the Brisbane system by an old Aborigine, as the cod found in the western rivers (McMinn, 1970). There were numerous reports about the relative abundance of what were presumed to be Murray cod in the upper Stanley River, a major tributary of the Brisbane River, during the early 1900s (Stephenson, 1953) and cod were so plentiful in the Brisbane and Mary systems during the early 1900s that some settlers used them as pig feed (J. Winkel, personal communication).

There has been a dramatic reduction in the distribution and abundance of cod in the eastern drainage and cod were probably extinct in the Brisbane and Richmond systems by the end of the 1930s. Only a few cod were reported from the Stanley River in the 1940s (Stephenson, 1953) and there has not been an authenticated report of cod from any location in the Brisbane system since that time (S.H. Midgley, R. McKay, personal communication). A marked decline of cod in the Richmond River occurred after 1926 when the north coast railway, which closely follows the river, was constructed north of Casino. Elderly, local residents claim that the river was extensively dynamited during the construction of the railway (B. Wilson, personal communication), and this, plus several very large fish kills involving many cod during heavy flooding in the 1920s and 1930s (D. Machin, S. King, personal communication) were probably the major factors in the demise of cod in this system. The last authenticated capture of a cod from the Richmond system was in 1971 (J. Bell, personal communication).

There has been a decline in the number of cod in

the Mary system since the early 1900s (S.H. Midgley, J. Winkel, K. Passlow, personal communication) and the population in this system is now restricted mainly to Six-mile, Tinana, Coondoo, Widgee, Station and Oby Oby Creeks. Cod are not common in the Mary River where the clearing of timber from the river and its banks for agricultural development has led to extensive siltation and loss of in-stream cover (S.H. Midgley, personal communication).

Cod disappeared from much of the Clarence system after a number of massive fish kills during the late 1920s and the 1930s. Long dry periods, followed by extensive bushfires and then heavy summer rains preceded some of the fish kills (S. Adams, K. Murphy, F. Winters, personal communication). Such conditions have been reported to cause a deterioration of water quality, including a marked depletion of dissolved oxygen, and increased turbidity, concentration of suspended solids, electrical conductivity and nutrient levels in some Australian rivers (Chessman, 1986). In addition, releases of contaminated water from the tailings dams at gold and tin mines are also thought to have caused the death of cod and other freshwater fish in parts of the Clarence system (F. Winters, personal communication).

Cod are now very rare or absent in the major northern tributaries of the system, the Clarence, Rocky and Cataract Rivers and they are no longer found in the Orara River, where they were once very common (Wilcox, 1863). The north coast railway, in part, closely follows the Orara River from Coffs Harbour to Grafton and dynamiting may have also been responsible for the rapid decline of cod in this river. Parts of the catchments of these rivers have undergone some agricultural development and this may have contributed to a loss of cod habitat. Since the late 1960s small numbers of cod (usually less than 5 kg) have been caught
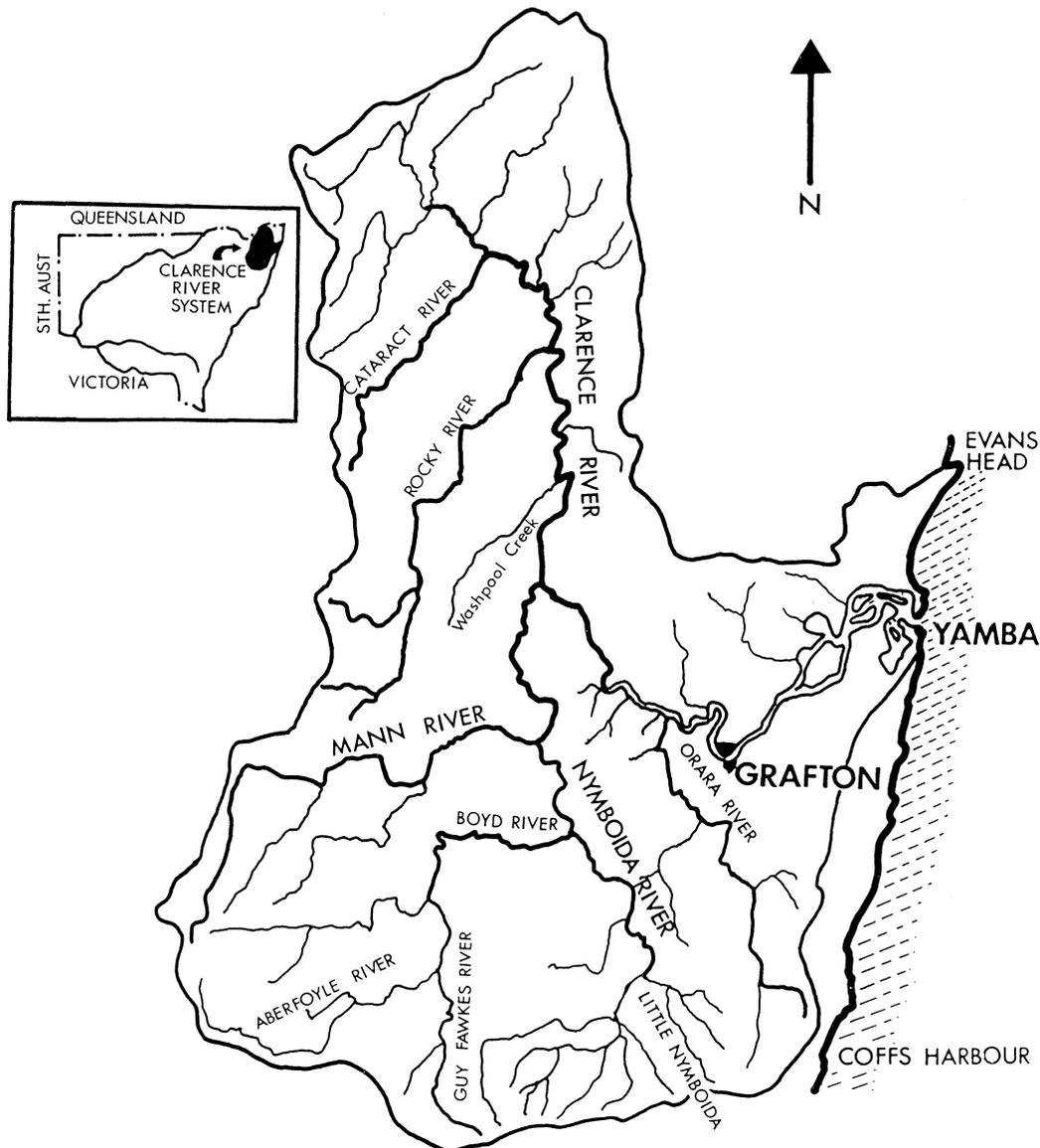

Fig.8. The Clarence River system, NSW.

only from tributaries such as the Nymboida, Little Nymboida, Boyd and Mann Rivers (Fig.8), where some pristine habitat still exists.

Maccullochella ikei and *M. p. mariensis*, like other species of *Maccullochella*, are large, long-lived, slow-growing species, with relatively low fecundity and a long generation period (Rowland, 1985). An extended age distribution in such fishes represents an evolutionary adaptation by which potentially high larval mortality in unfavourable breeding seasons is compensated for by repeated annual spawnings over the life span of individuals (Giesel, 1976; May, 1976). It is possible that *M. ikei* and *M. p. mariensis* have lost this advantage and that the intrinsic biology of these species has prevented their recovery from the low population levels caused by the fish kills and other factors. In addition, these species are potentially threatened by overfishing, pollution, habitat destruction or degradation, including the deterioration of water quality, the introduction of fishes not native to the eastern drainage (including possible hybridisation with hatchery-reared Murray cod), disease, the loss of genetic variability, and inbreeding. Small, isolated populations are extremely susceptible to extinction (Frankel & Soulé, 1981).

Maccullochella ikei, **an endangered species.** The eastern freshwater cod is now classified as endangered (Harris, 1987; Ingram *et al.*, 1990). In an effort to conserve *M. ikei*, there is a prohibition on the capture of freshwater cod, *Maccullochella* spp. from waters north of the Macleay River to the Queensland border, and the Eastern Freshwater Fish Research Hatchery has been constructed at Grafton so that techniques for the artificial breeding of *M. ikei* can be developed. Guidelines for the genetically-sound production and stocking of Australia's endangered and threatened fishes have been established (Rowland & Barlow, 1988) and the successful restocking of the Richmond and Clarence Rivers systems with hatchery-reared fry will reduce the vulnerability of *M. ikei* to the factors which cause extinction.

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APPENDIX

Table 1. Number and standard length of cod from each sampling site.

River system	Sampling sites	No. of cod sampled	Standard length range (mm)
Murray-Darling	Gwydir River (near Bingara) and MacIntyre River (Elsmore)	10	375-495
	Murray River (Lock 15), Edwards River (Stevens Weir) and Wakool River (Wakool)	15	355-520
	Farm dams near Narrandera	12	242-510
	Lake Mulwala	8	530-680
Clarence	Within 32 km of the junction of the Mann and Nymboida River	18	212-515
	as above	14[a]	305-456
Mary	Six-mile, Coondoo and Tinana creeks	9	235-555

[a] lectotype, paralectotypes and other material.

Table 2. Details of electrophoretic systems.

System	Medium	Buffer	Running conditions		
			voltage (V)	current (mA)	time (h)
A	polyacrylamide	discontinuous LiDH[a]	1500	40	3
B	starch	Tris maleate (pH 7.4)	50	40	15.5
C	starch	as above	60	50	6.5
D	starch	Tris borate EDTA (pH 8.6)	100	25	19
E	starch	Lithium borate-tris citrate (pH 8.2; pH 6.6)	220	–	3
F	cellulose acetate	Tris borate EDTA (pH 8.6)	200	–	1.5

[a] after Keenan & Shaklee (1985).

Table 3. Proteins assayed, tissues examined, the number of loci found and the number of cod examined in the electrophoretic analysis. E.s. = Electrophoretic system; *M.p.p.* = *Maccullochella peelii peelii*; *M.i.* = *Maccullochella ikei*; *M.p.m.* = *Maccullochella peelii mariensis*.

Protein	EC No.	Abbreviation	E.s.	Tissue	No. loci	No. cod analysed		
						*M.p.p.**	*M.i.*	*M.p.m.*
Phosphoglucomutase	5.4.2.2	PGM	B	Muscle	1	8	6	6
Fumarase	4.2.1.2	FUM	C	Muscle	1	8	6	6
Lactate dehydrogenase	1.1.1.27	LDH	C	Muscle	1	8	6	6
Malate dehydrogenase	1.1.1.37	MDH	C	Muscle	2	8	6	6
Aldolase	4.1.2.13	ALD	F	Liver	1	5	5	–
Superoxide dismutase	1.15.1.1	SOD	D	Liver	1	8	4	8
Aspartate aminotransferase	2.6.1.1	AAT	D	Liver	2	8	4	8
Glutamate dehydrogenase	1.4.1.3	GLUDH	F	Liver	1	5	5	–
Mannose-6-phosphate isomerase	5.3.1.8	MPI	B	Muscle	1	13	12	6
Isocitrate dehydrogenase	1.1.1.42	IDHP	B	Muscle	2	13	12	–
Malate dehydrogenase (NADP$^+$)	1.1.1.40	MDH(NADP)	E/F	Muscle	1	15	8	8
L-iditol dehydrogenase	1.1.1.14	IDDH	F	Liver	2	11	11	6
Glucose-6-phosphate isomerase	5.3.1.9	GPI	C	Muscle	2	13	11	6
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	D/E	Muscle	1	5	4	6
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH	F	Liver	2	9	7	4
Esterases	3.1.1.2	EST	B	Liver	?	5	5	–
General protein	–	–	A	Muscle	?	12	5	8

* includes specimens from each sampling site.

Table 4. Allele frequencies at loci of diagnostic enzymes in species of *Maccullochella*.

Locus	Allele	*M. p. peelii*	*M. ikei*	*M. p. mariensis*
Mpi-1	100	1.00	–	1.00
	87	–	1.00	–
Idh-1	100	1.00	1.00	1.00
Idh-2	100	–	1.00	no data
	75	1.00	–	
Iddh-1	100	1.00	–	0.17
	88	–	1.00	0.83
Iddh-2	100	1.00	1.00	1.00
Gpi-A$_2$	100	1.00	–	1.00
	92	–	1.00	–
G3pdh-1	100	–	1.00	–
	90	1.00	–	1.00
G6pdh-1	100	0.14	0.32	0.44
	92	0.38	0.05	–
	83	0.05	0.32	0.33
	69	0.38	–	0.22
	58	0.05	0.32	–

Table 5. Coefficients of genetic identity (I) and the genetic distances (D) between species of *Maccullochella*.

Cod	I	D
M. p. peelii; *M. ikei*	0.72	0.33
M. p. peelii; *M. macquariensis*	0.79[a]	0.24[a]
M. p. peelii; *M. p. mariensis*	0.85	0.16
M. ikei; *M. p. mariensis*	0.73	0.32

[a] Data from MacDonald (1978).

Table 6. Morphometric characters of *M. p. peelii* from Lake Mulwala.

Character	No.	Range	Mean	S.D.	t
% STANDARD LENGTH					
body depth I	6	30.3-37.0	32.2	2.50	4.22**
body depth II	5	28.5-29.5	28.8	0.43	0.68
peduncle depth	8	11.7-13.7	12.5	0.70	4.08**
peduncle length	8	20.3-23.3	21.5	0.92	0.69
predorsal length	8	40.8-43.5	42.1	0.91	3.53*
dorsal base	8	44.5-47.1	46.5	0.89	2.92*
anal base	8	16.1-18.7	17.5	0.77	1.11
pectoral fin	8	16.3-17.7	16.7	1.14	0.50
pelvic fin	8	13.0-15.5	14.6	0.76	0.82
head length	8	31.5-35.0	32.9	1.04	2.44
% HEAD LENGTH					
head width	3	47.0-51.9	48.9	2.67	3.14*
snout length	8	27.0-28.8	28.0	0.63	3.09*
post-orbital length	8	57.5-64.6	62.3	2.23	1.47
upper jaw length	8	43.0-46.5	45.3	1.24	0.10
inter orbital width	8	22.8-29.4	26.4	2.26	0.36
inter nare width	8	22.3-24.2	23.4	0.68	1.61
MERISTICS					
dorsal spines	8	11-12	11.4	0.52	0.94
dorsal rays	8	14-16	14.9	0.64	2.05
anal rays	8	12-13	12.6	0.52	0.31
pectoral rays	8	19-20	19.6	0.52	1.71
gill rakers	7	16-18	17.3	0.76	1.95
lateral line scales	6	72-86	79.3	6.38	3.76**
scale rows above	6	23-26	24.3	1.03	1.21
scale rows below	6	32-38	35.3	2.50	0.78
pyloric caecae	7	4	4.0	–	0
caudal rays	8	17-19	17.9	0.84	0.57

t – Student's t-test comparison of means with *M. p. peelii* from rivers and farm dams.
** significant P<0.001; * significant P<0.01; others not significant P>0.01.

Table 7. Comparison of morphometric characters between *M. p. peelii* (ex rivers and farm dams), *M. ikei* and *M. p. mariensis*.

Character	M. peelii					M. ikei					M. p. mariensis				
	No.	Range	Mean	S.D.	t^a	No.	Range	Mean	S.D.	t^b	No.	Range	Mean	S.D.	t^c
% Standard Length															
body depth (I)	22	27.2-31.6	29.1	1.29	0.30	4	27.3-30.2	28.9	1.23	1.85	9	28.3-33.1	30.5	1.49	2.57
body depth (II)	32	25.3-33.8	28.1	2.08	0.98	16	26.1-30.9	28.7	1.74	1.32	9	27.1-32.8	29.8	2.17	2.08
peduncle depth	37	10.3-13.0	11.4	0.66	0.39	18	10.7-12.6	11.5	0.55	7.35**	9	12.3-14.2	13.2	0.59	7.33**
peduncle length	37	19.8-23.3	21.8	1.00	0.94	18	20.0-24.8	22.1	1.36	3.33*	9	19.0-21.4	20.4	0.69	3.77**
predorsal length	36	36.8-42.5	40.3	1.35	0.04	18	36.7-43.1	40.2	1.86	0.44	9	38.5-43.1	40.5	1.34	0.58
dorsal base	35	42.4-47.9	45.1	1.29	3.23*	18	40.1-47.2	43.7	1.81	2.81*	9	44.1-47.2	45.5	0.85	0.89
anal base	37	16.4-21.0	17.9	1.02	5.40**	18	15.1-17.5	16.4	0.65	1.08	9	14.7-18.6	16.9	1.32	2.50
pectoral fin	37	14.7-18.1	16.8	0.81	2.96*	17	15.8-19.0	17.5	0.79	0.14	9	16.5-19.3	17.5	0.94	2.12
pelvic fin	37	13.2-16.4	14.9	0.86	13.49**	17	16.9-19.5	18.4	0.90	0.41	9	16.4-19.8	18.2	1.19	9.85**
dorsal spine (5th/6th)	37	6.5-12.5	8.7	1.64	3.33*	17	8.5-13.0	10.2	1.17	4.02**	9	6.1-9.8	8.3	1.04	0.68
head length	37	30.3-34.5	32.0	0.98	2.97*	18	31.7-36.7	32.9	1.22	0.39	9	31.8-34.3	33.0	0.75	3.11*
% Head Length															
head width	29	40.9-68.0	57.4	4.45	0.08	15	46.0-63.5	57.3	5.24	0.14	9	47.8-64.6	57.6	5.57	0.12
snout length	40	26.5-30.5	29.0	0.91	0.98	18	27.9-30.6	29.3	0.87	0.36	9	27.8-30.3	29.2	0.86	0.24
post-orbital length	39	56.9-64.9	61.3	1.71	7.50**	18	54.7-59.7	57.9	1.31	3.94**	9	57.8-61.5	59.9	1.12	2.40
orbit	25	9.7-12.9	10.9	0.67	11.56**	18	12.8-19.0	14.6	1.41	4.66**	9	10.0-13.8	12.0	1.23	3.46*
upper jaw length	40	41.0-48.4	45.2	1.84	2.54	18	42.8-48.9	46.5	1.56	1.24	9	38.9-49.3	45.5	2.91	0.28
inter orbital width	39	23.6-29.6	26.7	1.67	4.40**	18	20.8-27.5	24.6	1.57	4.54**	9	25.1-30.4	27.7	1.88	1.62
inter nare width	39	21.0-24.9	22.8	0.96	0.23	17	21.4-24.4	23.0	0.72	1.24	9	22.4-25.3	23.4	1.02	1.69
Meristics															
dorsal spines	37	11-12	11.2	0.42	1.14	18	10-12	11.1	0.64	0.27	9	11	11.0	–	1.57
dorsal rays	36	14-16	15.3	0.54	3.33*	18	14-16	14.8	0.52	0.97	9	15	15.0	–	1.83
anal rays	37	12-15	12.5	0.77	5.50**	18	11-12	11.4	0.51	4.24**	9	12-13	12.3	0.50	0.78
pectoral rays	37	18-21	19.1	0.91	3.67**	18	16-19	18.2	0.79	3.39*	9	18-20	19.2	0.67	0.53
gill rakers	32	16-20	18.1	1.03	1.13	14	18-20	18.4	0.65	0.42	8	17-21	18.6	1.60	1.17
lateral line scales	35	66-80	72.1	3.95	1.08	18	65-82	73.4	4.72	1.73	9	68-74	70.6	2.30	1.12
scale rows above	37	22-34	25.9	3.04	2.79*	17	19-28	23.5	2.65	0.82	9	23-26	24.2	1.09	1.60
scale rows below	37	29-50	37.4	6.37	1.81	18	32-52	40.5	4.81	4.89**	9	30-34	32.4	1.51	2.31
caudal rays	36	15-20	17.6	1.10	0.57	17	17-19	17.5	0.72	1.29	9	16-18	17.1	0.60	1.40

t – Student's t-test; t^a – *M. p. peelii* vs *M. ikei*; t^b – *M. ikei* vs *M. p. mariensis*; t^c – *M. p. peelii* vs *M. p. mariensis*.
** significant $P<0.001$; * significant $P<0.01$; others not significant $P>0.01$.

Table 8. Osteological characters of *Maccullochella* spp. as determined from x-rays. Number of specimens examined in parentheses.

Character	M. p. peelii (3)	M. ikei (4)	M. p. mariensis (5)	M. macquariensis (3)
predorsal bones	3-4	2-3	2-3	2-3
precaudal vertebrae	15	15	15	14
caudal vertebrae	20	20	19-20	21
first anal pterygiophore[a] (%)	86-97	89-97	79-86	74-79

[a] length of first anal pterygiophore expressed as a percentage of the distance from the base of this pterygiophore to the ventral surface of the first caudal vertebra.

Table 9. Results of the cross-breeding experiment between Murray cod (MC) and eastern freshwater cod (EFC). Milt from two male Murray cod was used to fertilise the eggs stripped from the three females which ovulated.

Female	Weight (kg)	Oocyte diameter before injection (mm)	HCG (IU/kg)	Latent period (h)	Ovulation	No. of eggs	No. of larvae	Hatch rate (%)
EFC	1.35	2.6	500	48	–			
				55	–			
				68	–			
EFC	3.8	3.0	1000	47	–			
				48	–			
				56	–			
				68	–			
EFC	2.15	2.8	2000	46	+	6,750	147	2
				48	+	1,900	630	33
						8,650	777	9
MC	11.0	2.9	1000	48	+	27,200	675	3
				50	+	22,500	16,785	75
						49,700	17,460	35
MC	11.6	3.0	1000	4 8	+	29,000	16,910	58
				49	+	17,500	15,755	90
						46,500	32,665	70

Table 10. Morphometric characters of the lectotype, paralectotypes, other material and other specimens of *Maccullochella ikei*.

Character	Lectotype	Range Paralectotypes (n=3)	Other material (n=10)	Other specimens (n=18)	*M. ikei* (n=32)
Standard length (mm)	410	385-503	305-456	212-515	
% STANDARD LENGTH					
body depth I	28.3	29.7-29.9	26.0-30.3	27.3-30.2*	26.0-30.3
body depth II	28.8	28.8-31.5	27.0-30.2	26.1-30.9*	26.1-31.5
peduncle depth	11.3	11.0-13.2	11.0-13.2	10.7-12.6	10.7-13.2
peduncle length	19.8	21.5-22.2	21.5-23.3	20.0-24.8	19.8-24.8
predorsal length	39.9	38.7-42.2	39.5-42.6	36.7-43.1	36.7-43.1
dorsal length	45.6	41.3-45.7	40.8-45.0	40.1-47.2	40.1-47.2
anal base	17.2	15.0-16.2	15.2-18.1	15.1-17.5	15.0-18.1
pectoral fin	16.7	17.4-18.1	14.2-18.5	15.8-19.0*	14.2-19.0
pelvic fin	17.9	17.2-19.4	16.6-19.7	16.9-21.0*	16.6-21.0
dorsal spine (5th/6th)	10.8	9.4-10.9	9.3-11.2	8.5-13.0*	8.5-13.0
head length	32.7	32.2-33.4	31.9-33.7	31.7-36.7	31.7-36.7
% HEAD LENGTH					
head width	62.1	59.3-65.9	58.8-66.2	46.0-63.5*	46.0-66.2
snout length	29.2	27.0-30.8	29.2-30.9	27.9-30.6	27.0-30.9
post-orbital length	57.1	56.2-57.5	56.6-59.4	54.7-59.7	54.7-59.7
orbit length	14.3	13.4-15.1	12.0-15.3	12.8-19.0	12.0-19.0
upper jaw length	47.1	47.4-47.7	40.3-51.1	42.8-48.9	40.3-51.1
inter orbital width	23.5	27.1-27.4	23.5-29.1	20.8-27.5	20.8-29.1
inter nare width	22.4	24.3-24.6	22.4-25.0	21.4-24.4*	21.4-25.0
MERISTICS					
dorsal spines	12	11-12	10-12	10-12	10-12
dorsal rays	14	13-15	13-15	14-16	13-16
anal rays	12	11	11-12	11-12	11-12
pectoral rays	17	17	17-19	16-19	16-19
gill rakers	–	–	–	18-20*	18-20
lateral line scales	70	68-71	65-80	65-82	65-82
scale rows above	23	24-25	25-30	19-28*	19-30
scale rows below	32	32-34	29-34	32-42	29-42
caudal rays	18	17-18	17-19	17-19*	17-19
predorsal bones	2	2-3	–	–	2-3
precaudal vertebrae	15	15	–	–	15
caudal vertebrae	20	20	–	–	20

* these data from 4-17 specimens (see Table 7).