

## ***Milyeringa veritas* (Eleotridae), a remarkably versatile cave fish from the arid tropics of northwestern Australia**

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### **Synopsis**

The blind cave gudgeon *Milyeringa veritas* is restricted to groundwaters of Cape Range and Barrow Island, northwestern Australia. It occurs in freshwater caves and in seawater in anchialine systems. It is associated with the only other stygobitic cave vertebrate in Australia, the blind cave eel, *Ophisternon candidum*, the world's longest cave fish, and a diverse stygofauna comprising lineages with 'tethyan' tracks and widely disjunct distributions, often from North Atlantic caves. The cave gudgeon inhabits a karst wetland developed in Miocene limestones in an arid area. There is an almost complete lack of information on the basic biology of this cave fish, despite it being listed as threatened under the Western Australian Wildlife Conservation Act. Allozyme frequencies and distributions indicate significant population sub-structuring on the Cape Range peninsula such that the populations are essentially isolated genetically suggesting that more than one biological species is present. Further, they suggest that the vicariant events may have been associated with a series of eustatic low sealevels. Analysis of intestinal contents indicates that they are opportunistic feeders, preying on stygofauna and accidentals trapped in the water, at least at the sites sampled which were open to the surface, a conclusion supported by the results of stable isotope ratio analysis. The gudgeons are found in freshwater caves and throughout deep anchialine systems in which they occur in vertically stratified water columns in which there is a polymodal distribution of water chemistries (temperature, pH, salinity, dissolved oxygen, redox, dissolved inorganic nitrogen series, hydrogen sulphide).

### **Introduction**

The arid tropical northwest of Australia is notable for the diversity of the subterranean fauna that inhabits the karstic Cape Range peninsula and Barrow Island. It includes a terrestrial fauna with wet forest affinities (Humphreys 1993a,b, 2000a,b), and an aquatic fauna with both limnic and anchialine components. The latter includes many disjunct Tethyan lineages (Humphreys 1993a, 2000b, Jaume & Humphreys 2001, Jaume et al. 2001) whose congeners or sister taxa inhabit caves on either side of the North Atlantic, in the Caribbean region and the Canary Islands (see below).

Included in this subterranean karst wetland (ANCA 1996, Humphreys 2000b) are the only known stygobiotic cave vertebrates in Australia. They are

the blind cave gudgeon, *Milyeringa veritas* Whitley, 1945 (Perciformes: Eleotridae) and the blind cave eel, *Ophisternon candidum* (Mees, 1962) (Synbranchiformes: Synbranchidae) (Figure 1) which occur sympatrically over much and possibly all of their ranges. The latter species is considered by Romero & Vanselow (2000b) and it will not be discussed further here. Globally, there are few occurrences of sympatric species of cave fishes (Thinès & Proudlove 1986). Proudlove<sup>1</sup> (personal communication) records 13 instances of two species and three instances of three species occurring in sympatry. Kuhajda & Mayden

<sup>1</sup>Proudlove, G.S. 1997. A synopsis of the hypogean fishes of the world. *In: Proceedings of the 12th International Congress of Speleology, La Chaux de Fonds, Switzerland, August 1997*, 3: 351–354.

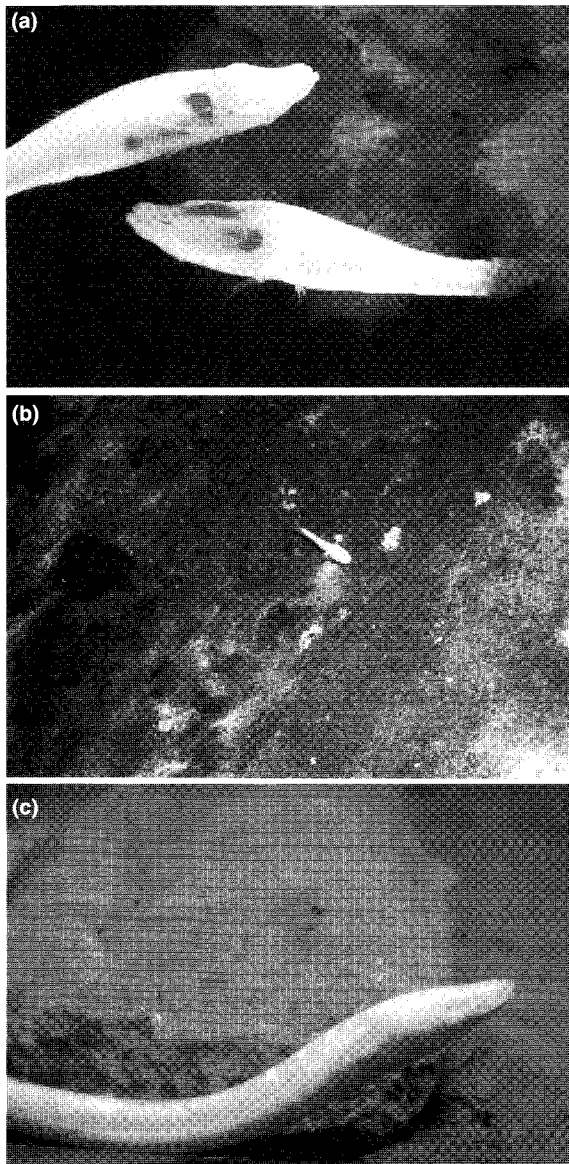


Figure 1. a – *Milyeringa veritas* from Kubura Well (photo: Douglas Elford, Western Australian Museum); b – *M. veritas* foraging over various sulphur bacteria in Bundera Sinkhole, an anchialine cave (photo: Stefan Eberhard, Western Australian Museum); c – *Ophisternon candidum* from Kubura Well (photo: Douglas Elford, Western Australian Museum).

(2001) report the first known occurrence of sympatry between North American cave fish species.

While some cave fishes, particularly the Mexican characid *Astyanax fasciatus*, and the American Amblyopsidae, have been extensively researched

(e.g. Wilkens 1988, Bergstrom 1997, review in Weber et al. 1998b), little is known of those inhabiting the Cape Range peninsula. This is partly because even the general affinities of *M. veritas* are unknown, precluding the interesting questions that can be posed about the evolution of those cave fishes that are part of lineages with extant epigeic sister taxa. The identification of the phyletic affinities of *M. veritas* would be the most effective information to stimulate research on the Australian cave fishes. *Milyeringa veritas* is the only stygobitic species of eleotrid known in the world (Romero & Vanselow 2000a) and occupies a wide range of physico-chemical environments (Humphreys 1999b). While no research has focused on these Australian cave fishes, a variety of data has been collected in association with other projects and a synthesis of this information is presented here to stimulate and facilitate research on this versatile species.

### History and affinities

*Milyeringa veritas* was described from subterranean waters of the Cape Range peninsula in the monotypic family Milyeringidae (Gobioidea) (Whitley 1945). Mees (1962) recognized the eleotrid affinities of *Milyeringa*, a family widespread in tropical and subtropical shallow marine to fresh waters mainly in the Indo-Pacific region (Nelson 1984). The monotypic genus *Milyeringa* is endemic to the Cape Range peninsula and Barrow Island but its phylogenetic relationships within the Eleotridae have not been established. *Milyeringa veritas*'s closest affinity is possibly with the eleotrid genus *Butis*, a mangrove dwelling genus of brackish and fresh waters of the Indo-Australian Archipelago (i.e. Malay Peninsula to northern Australia including Indonesia, Philippines and most of Melanesia) (G.R. Allen personal communication). Mangroves are still present on the Cape Range peninsula although they were formerly more widespread (Morse 1993).

On consideration of the geological and tectonic history of the area Humphreys & Adams (1991) suggested that a southern extension of the range of *M. veritas* was unlikely owing to the salinity of the groundwater but that 'it may be worth searching wider afield for this fauna ...' to the northeast (see also Humphreys 1993a, b). In 1996, elements of the tethyan crustacean fauna were found as predicted where the Precambrian plateau (the Western Shield,

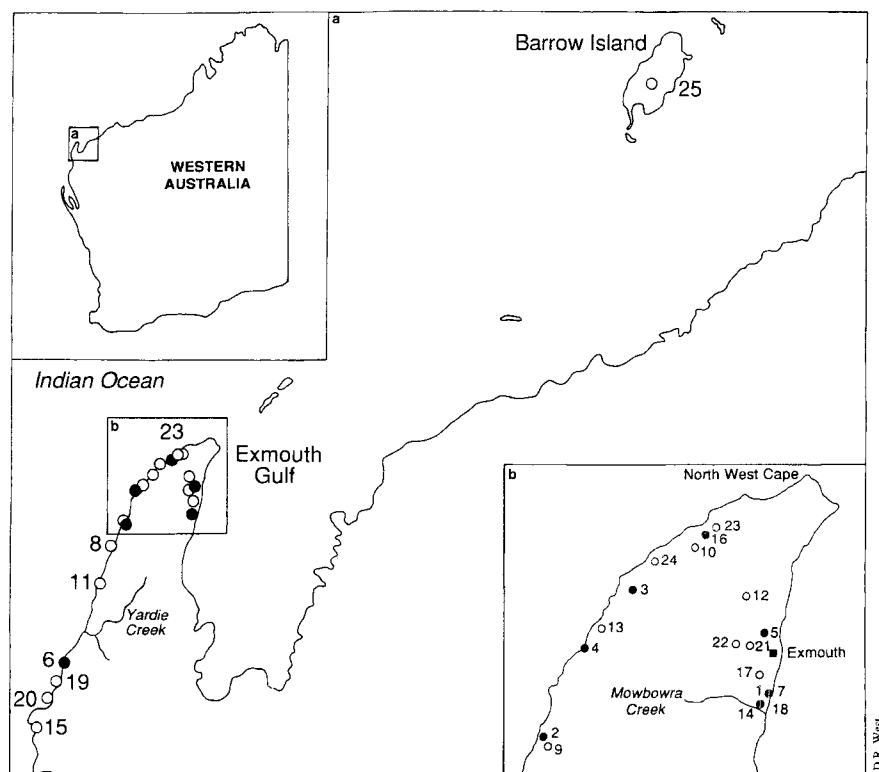


Figure 2. The distribution of blind cave fishes in Australia. Filled circles denote *Ophisternon candidum*. *Milyeringa veritas* is present at all locations denoted by open or filled circles except location 4 (Tantabiddi Well). Top of the map is north.

Beard 1998) approaches the shore line to the south of Karratha, and in 1999 *M. veritas* itself was collected from deep groundwater on Barrow Island (Humphreys 1999c).

Barrow Island lies on the shallow North West Shelf (Figure 2) which, until the early Holocene, ca. 8000 years ago, would have been connected by land to the Cape Range peninsula owing to the generally lower sea level over much the last million years (Chappell & Thom 1977). Hence, for much of that time there would have been a broad karstic plain, encompassing the North West Shelf from the tip of the peninsula to the Fortescue River and including Barrow Island, that would have allowed widespread dispersal of the subterranean fauna (Humphreys 1993b). Indeed, both terrestrial (Harvey & Humphreys 1995) and aquatic (Adams & Humphreys 1993, Humphreys 2000a) con-specific subterranean fauna occur on Barrow Island and the northeast coast of the Cape Range peninsula (Humphreys 1993b).

## Regional context

### Distribution

The known distribution of *M. veritas* is shown in Figure 2 and details of the sites and the history of their discovery are given in Humphreys (1999c). The species' range encompasses the coastal plain from Exmouth to Jarvis Well (location 15), a distance along the coast of about 136 km, and one site on Barrow Island which lies 160 km to the northeast of North West Cape across the shallow North West Shelf.

There has been extensive sampling for stygofauna along the coast of both the mainland and the Cape Range peninsula, as well as from Barrow Island<sup>2</sup> (Pesce et al. 1996, figure 29, W.F. Humphreys unpublished

<sup>2</sup> Humphreys, W.F. 1994. The subterranean fauna of the Cape Range coastal plain, northwestern Australia. Report to the Australian Heritage Commission and the Western Australian Heritage Committee. 202 pp.

data) and the species appears to be endemic to the Cape Range and Barrow Island karst formed on anticlines of Tertiary limestones (Wyrwoll et al. 1993, McNamara & Kendrick 1994). However, *M. veritas* has rarely, and *O. candidum* has never been collected using methods not involving direct sighting prior to capture, hence the mainland sampling, which was conducted in bores and pastoral wells using haul nets and traps without visual contact, could have missed them. It is notable that other elements of the Cape Range and Barrow Island stygofauna do occur on the mainland in the calcrete and alluvial fans near the mouths of the Robe and Fortescue rivers (Humphreys 1999a). One tethyan element, the thermosbaenacean *Halosbaena*, has even been recorded from an altitude of 300 m in the Robe Valley, the approximate high sea level stand in the Late Eocene on the Yilgarn (G.W. Kendrick personal communication 1999).

#### *Geology and geomorphology*

The Cape Range peninsula comprises an anticline of marine limestones of Late Eocene–Miocene age rising to an altitude of 314 m. The peninsula is fringed by a coastal plain interrupted on the west coast by a series of erosion terraces cut into the Tertiary limestones during sea level stadia and overlain in places by Quaternary carbonates forming a karst pavement except where they are overlain by Pleistocene and Holocene dunes (Wyrwoll et al. 1993). The western shore is bordered by the Ningaloo Reef, the longest fringing reef in Australia, and considered an integral part of the Cape Range karst province<sup>3</sup>. Further information on the geology and hydrogeology may be sought in Allen (1993).

#### *Climate and vegetation*

The area is in the arid tropics with high evaporation (mean 3219 mm) and receives its rainfall (median 280 mm) as a result of several meteorological processes (Beard 1975, Gentilli 1972, 1979). The predominantly episodic heavy rainfall may occur through much of the year but with very low predictability (Humphreys et al. 1989) and it is responsible for the principal recharge of

the freshwater aquifers through the surface karst which affects the elevation of the groundwater surface for prolonged periods. Temperatures are high (mean 27°C) and the mean monthly maximum temperature exceeds 35°C for four months of the year.

On the coastal plain the vegetation is naturally dominated by tussock grasses with eucalypt shrubs and *Ficus* associated with caves but which occurs more widely on the karst. Further details on the flora and vegetation associations of the range can be found elsewhere<sup>4</sup> (Beard 1975, Keighery & Gibson 1993).

#### *Regional groundwater*

The general hydrogeological model for oceanic islands has been applied to the groundwater of Cape Range peninsula (Allen 1993). Following the Ghyben–Herzberg principle (Ford & Williams 1989), a freshwater lens overlies saltwater so that locally a wedge of saltwater intrudes under the freshwater contained in the limestone, and between which occurs a zone of mixing that broadens towards the coast. In the Exmouth area, on the northeastern part of the Cape Range peninsula, the freshwater–saltwater transition, at about 5 km from the coast, is exceptionally far inland. In the Exmouth area the inland limit to the salt-water interface appears to be controlled by the presence of solution cavities and channels below the water table (Martin 1990). Such aquifers are vulnerable to saltwater upconing as a result of pumping water from the thin freshwater lens, and to the inland migration of seawater.

#### **Habitat**

##### *General*

*Milyeringa veritas* has been recorded exclusively from inland karst systems from within 150 m of the coast and up to 4.3 km inland. They are found in freshwater caves as well as in anchialine ecosystems; the latter comprise fresh to brackish groundwaters overlying seawater that has no surface connection to the sea but which is affected by marine tides (Sket 1981, 1986, 1996, Stock

<sup>3</sup> Hamilton-Smith, E., K. Kiernan & A. Spate. 1996. Karst management considerations for the Cape Range karst province, Western Australia. W.A. Department of Environmental Protection, Perth. 80 pp.

<sup>4</sup> Payne, A.L., P.J. Curry & G.F. Spencer. 1987. An inventory and condition survey of rangelands in the Carnarvon Basin, Western Australia. Western Australian Department of Agriculture Technical Bulletin No. 73. 478 pp.

et al. 1986). Much of the habitat of *M. veritas* is known to be affected by marine tides (Humphreys et al. 1999, figure 1) but the tidal influence is much more marked on the west coast of the peninsula (W.F. Humphreys & R.D. Brooks unpublished).

*Milyeringa veritas* may be found swimming in sunlight at the surface in small karst windows in the anchialine system and at depths of up to 33 m in caves containing seawater (Humphreys 1999b). It has been sampled from groundwaters through bore holes in which the water table is up to 47 m below the ground and remote from known openings to the surface.

### Physico-chemical environment

Humphreys & Adams (1991) and Humphreys<sup>2</sup> reported the occurrence of *M. veritas* from a range of water chemistries (Table 1) and subsequently from more saline waters (Yager & Humphreys 1996) with complex physico-chemical stratification (Humphreys 1999b, Humphreys et al. 1999). *M. veritas* populations are

known from freshwater caves (0.3 g l<sup>-1</sup> TDS: Poore & Humphreys 1992) but also inhabit the anchialine system with salinities up to 34 g l<sup>-1</sup> TDS (Yager & Humphreys 1996, Humphreys 1999b) – they are reported to be susceptible in captivity to fungus infections (*Saprolegnia*) in freshwater (Young 1986). The fish occur in hyperoxic surface water to suboxic (see Sket 1996) waters below the pycnocline (Humphreys 1999b) and they have been seen foraging immediately above sediments (Figure 1) covered by black and white sulphur bacteria.

At one location, Bundera Sinkhole, *M. veritas* inhabits an anchialine cave with marked physico-chemical and biological stratification (Figure 3). The fish occur below multiple layers of hydrogen sulphide and redox and oxygen minima associated variously with sulphur bacteria and a cascade of dissolved inorganic nitrogen series (Humphreys 1999b). While *M. veritas* inhabits waters with a remarkable range of physico-chemical characteristics (Yager & Humphreys 1996), it is not known how rapidly they move between these different water masses: the same individuals could move

Table 1. Water analysis for the sites inhabited by *Milyeringa veritas* ( $n = 20$ ). The data for individual sites are contained in Humphreys<sup>2</sup>. All units are in mg l<sup>-1</sup> unless otherwise stated.

Parameter	Mean	SE	Min	Max	CV
pH	7.45	0.06	7.02	8.08	3
Turbidity NTU	3.83	1.00	0.6	16	116
Colour TCU	4.7	1.7	0	34	162
Conductivity mS m <sup>-1</sup>	1295	307	35	4700	106
TFS	8168	1930	250	26 500	106
TFS-CO <sub>2</sub>	8013	1927	210	26 500	107
Fe-unfiltered	0.20	0.08	0	1.6	185
Mn-unfiltered	0.04	0.02	0	0.45	256
Al	0.12	0.03	0.02	0.6	119
Na	2352	58 948	7780	112	
K	94.0	22.7	2	310	108
Ca	161	22.3	15	390	62
Mg	288	68.7	3.4	940	107
Hardness as CaCO <sub>3</sub>	1580	336	50	4840	95
Alkalinity	4.5	0.34	1.6	8.2	34
Cl	4321	1088	54	15 050	113
SO <sub>4</sub>	579	145	7	1950	112
Si – as SiO <sub>2</sub>	13.8	0.9	2.8	23	29
Filterable organic carbon	19.6	4.1	1	74	93
Total phosphorous	0.05	0.01	0.009	0.185	91
Free reactive phosphorous	0.026	0.007	0.004	0.13	121
Total Kjeldahl nitrogen	0.36	0.09	0.04	1.7	113
Nitrite as nitrogen	0.02	0.009	0	0.19	202
Nitrate as nitrogen	3.2	1.54	0.02	31.5	215
Ammonia as nitrogen	0.06	0.02	0	0.36	135

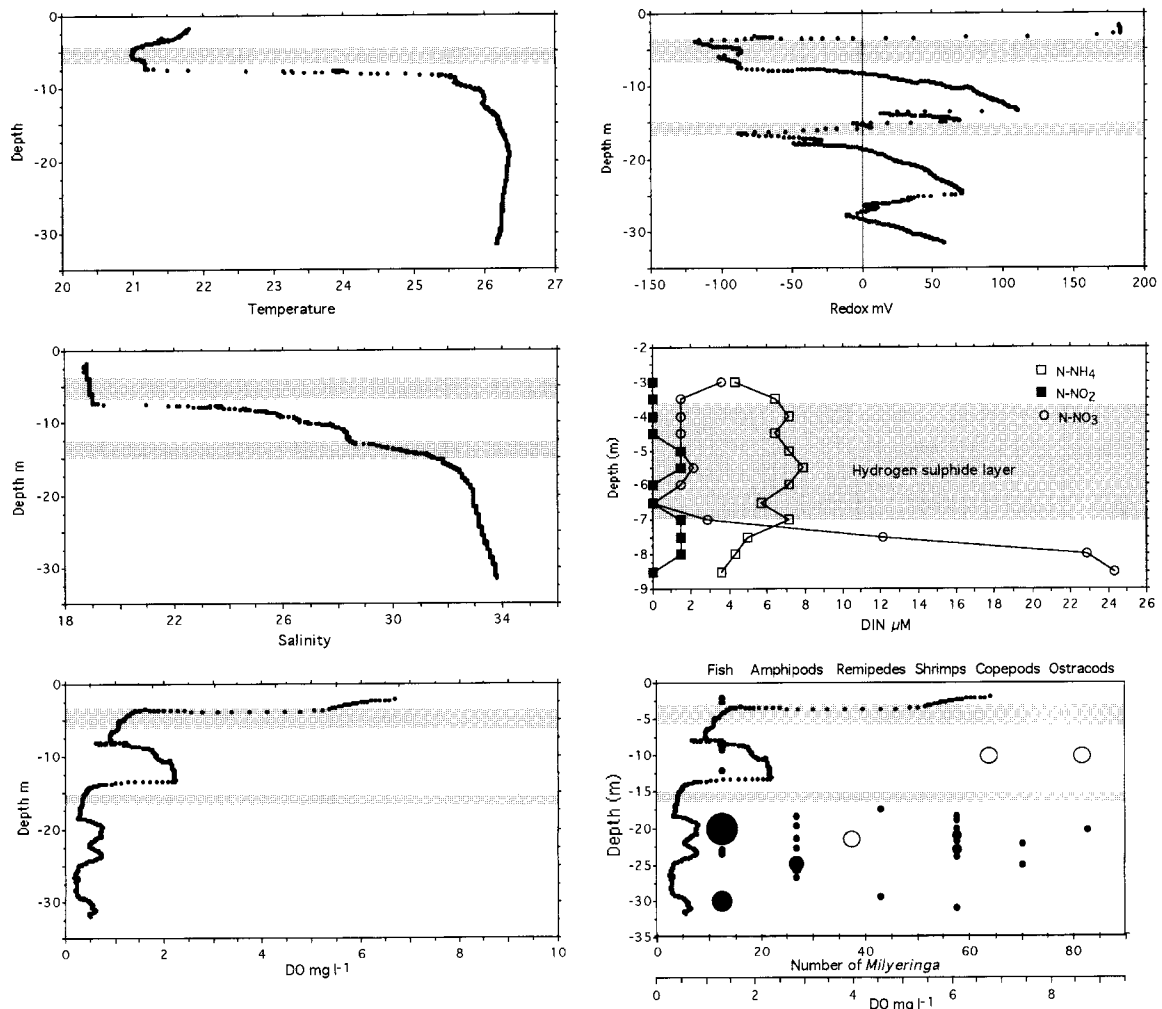


Figure 3. Vertical profile with depth in Bundera Sinkhole in 1997 where the grey bands denote the zones of elevated hydrogen sulphide concentration (see Humphreys 1999b: Figure 2f): (a) temperature ( $^{\circ}\text{C}$ ), (b) salinity ( $\text{g l}^{-1}$  TDS), (c) dissolved oxygen ( $\text{mg l}^{-1}$  DO), (d) oxidation reduction (redox) potential (mV), (e) dissolved inorganic nitrogen species (DIN  $\mu\text{M}$ ) N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, (f) vertical distribution of sightings of the fauna in Bundera Sinkhole reported by divers [(a) the closed circles denote the vertical distribution of the taxon named at the head of the column, (b) the open circles denote the number of blind gudgeons, *Milyeringa veritas*, counted at one occasion at each of three depths (second bottom scale), (c) the curve denotes the DO concentration (bottom scale)]. These data are illustrative only as no regular sampling has been conducted through time. Further details in Humphreys (1999b) and Humphreys et al. (1999).

regularly or rarely between the water types, there may be life interval movements or different stocks may inhabit the different water masses.

#### Associated fauna

*Milyeringa veritas* is part of a rich stygofaunal community that contains a number of ecogeographic relicts and phyletic relicts with tethyan affinities<sup>2</sup> (Humphreys

1993a, 2000a, Danielopol et al. 2000). These include the only member of the class Remipedia (Yager & Humphreys 1996) and the order Thermosbaenacea (Poore & Humphreys 1992) known from the southern hemisphere which are congeneric with species occurring in caves on either side of the North Atlantic.

In the general groundwater of the Cape Range peninsula both epigeal and stygal taxa cohabit with *M. veritas*. The stygal species *Halosbaena tulki* Poore &

Humphreys (Thermosbaenacea), *Haptolana pholeta* Bruce & Humphreys (Isopoda: Cirolanidae) and *Nedsia douglasi* Barnard & Williams occur in freshwater. *Stygiocaris lancifera* Holthuis and *S. stylifera* Holthuis (Malacostraca: Atyidae) are found in freshwater and anchialine systems. *Grandidierella* sp. nov., a non-stygial species, occurs in open anchialine pools in which *M. veritas* may occasionally forage. A number of the sites with *M. veritas* are also the type localities for other cave restricted species, some of which are prey of the fish, such as Thermosbaenacea and atyid shrimps (Humphreys & Feinberg 1995).

On Barrow Island *M. veritas* is known from a single bore that also contains calanoid, cyclopid and harpacticoid copepods including *Inermipes humphreysi* Lee & Huys (Harpacticoida: Ameiridae), *Halosbaena tulki* (Thermosbaenacea), the amphipods *Nedsia sculptilis* Bradbury & Williams (Melitidae) and *Bogidomma australis* Bradbury & Williams (Bogidiellidae), *Haptolana pholeta* (Cirolanidae) and *Stygiocaris stylifera* (Atyidae).

The vertical stratification of the faunal associations is best documented from Bundera Sinkhole because it is accessible by divers to a depth of 33 m and *M. veritas* occurs through the full depth of the water column (Figure 3f). Bundera Sinkhole is the only deep anchialine system known in Australia and the only known continental anchialine system in the southern hemisphere (see Iliffe 2000, figure 1) as well as the only known site for the crustacean class Remipedia in the southern hemisphere (Yager & Humphreys 1996). It is the most complex habitat known to be inhabited by *M. veritas*. Several taxa are known only from below the thermohalocline in the cave, namely *Lasionectes exleyi* Yager & Humphreys (Remipedia), *Danielopolina kornickeri* Danielopol et al. 1999 (Ostracoda: Halocyprida: Thaumatoocyprididae), *Liagoceradocus branchialis* Bradbury & Williams (Crustacea: Hadziidae), *Stygiocaris stylifera*, and the copepods *Bunderia misophaga* Jaume & Humphreys, 2001 (Calanoida: Epactericidae), *Stygocyclopia australis* Jaume et al., 2001 (Calanoida: Pseudocyclopididae) and *Speleophria bunderae* Jaume et al., 2001 (Misophrioida: Speleophriidae).

Above the thermohalocline in the photic zone this anchialine system also contains a number of non-stygial plants and animals (the algae *Rhizoclonium tortuosum* (Dillwyn) (Chlorophyta: Cladophoraceae) and *Lamprothamnium papulosum* J. Groves (Charophyta: Characeae), and the invertebrates *Prionospio*

sp. nov. (Spionidae: Polychaeta), *Halicyclops longifurcatus* Pesce et al. (Copepoda: Cyclopididae), ostracods, *Iravadia* sp. (Mollusca: Iravadiidae, Slack-Smith 1993), water striders (Gerridae: Hemiptera) and *Kiefferulus intertinctus* Skuse (Chironomidae, Diptera). A fuller description of the epigeal fauna associated with these karst wetlands is given elsewhere (Humphreys 2000a,b).

Although no systematic sampling has been conducted, it is clear that both the distribution and abundance of the fauna, including *M. veritas*, differs between years (Humphreys 1999b). While *M. veritas* has been recorded throughout the water column, the vertical distribution differs markedly between years. In 1994, there was a large concentration of the fish at the extreme depth of the cave, while they were entirely lacking from this area in 1997 when there were concentrations just below the pycnocline (ca. 10 m) and at ca. 20 m, between the H<sub>2</sub>S layers (Figure 3f). This change in location of the principle predator (Humphreys & Feinberg 1995), and of *D. kornickeri*, suggests that the ecosystem is variable through time.

Like *M. veritas*, other fishes with reduced or vestigial eyes, which are sometimes found in total darkness, are known to inhabit caves exhibiting a density interface (thermohalocline: sensu Humphreys 1999b). Species of the genus *Lucifuga* variously occur both above (Cohen & McCosker 1998) and below (Díaz Perez 1987b) the density interface at depths of up to 40 m (Díaz Perez 1987b), in freshwater (Díaz Perez 1987a) or saltwater (34 mg<sup>-1</sup>: Palmer 1985). They may also occur with relictual crustaceans (Yager 1981, Palmer 1985, Díaz Perez 1987a) such as Remipedia, Thermosbaenacea and *Thaumatoocypris* (Ostracoda: Yager 1981, Díaz Perez 1987a).

## Biology

### *Appearance and behaviour*

*Milyeringa veritas* is eyeless, depigmented and translucent, and *in vivo* the brain is visible through its braincase, the scales are reduced on the body and absent from the head, all indicative of a high degree of troglomorphy. The general morphology of *M. veritas* is convergent with other cave dwelling fish such as *Amblyopsis rosae*, the Ozark cave fish of Missouri (Bergstrom 1997). Although visible eyes are overtly lacking it is not known whether the eyes of *M. veritas* are entirely regressed as is suggested by the behavioural observation that the fish in caves show no rapid

response to sudden bright light; however, they sometimes swim in sunlight in anchialine pools. The degree of eye reduction in *M. veritas* does suggest that the lineage has been troglobitic for a long time since the degree of eye reduction may be considered indicative of the time since troglobite evolution (Wilkens et al. 1989). The latter correlated the degree of eye reduction in *Lucifuga* and *Ogilbia* with the phylogenetic age of the lineages in the New World tropics as deduced from geological and eustatic information.

*Milyeringa veritas* often hangs more or less motionless in the water or perches on rock ledges for long periods. When the water is disturbed the fish may swim directly to investigate the disturbance, gradually distance itself from the area, or swim to the bottom and move behind obstacles, especially to move beneath ledges. Despite the reported sluggish nature of these fish (Knott 1993), they may exhibit strong escape behaviour from a net, are strong swimmers for short distances, even leaping from the water and, if stranded, can flip their bodies into the air. Young (1986) reported that, in captivity, they displayed 'aggression' with the 'typical gill flaring and body waggle of a gudgeon' and that when familiar with their surroundings could be quite evasive.

In shallow conduits of the anchialine system that drain at low tide, *M. veritas* may be seen closely following the advancing tidal front, apparently foraging over the newly submerged substrate, an observation consistent with the apparent opportunistic intake of food revealed by analysis of the intestinal contents. Although *M. veritas* occur in particular parts of caves, often in some numbers (see below), they do not overtly group. They move widely through the water column within a given cave but it is not known whether individuals cross the pycnocline.

### Numbers

Several *M. veritas* are commonly seen in caves, but there are some sites where more than 100 may be seen (Humphreys 1999b). Formal determination of the number of *M. veritas* at any location is limited to Kubura Well, actually an open cave that is connected to an entirely flooded chamber extending to a depth of 11 m. The minimum size of the population of *M. veritas* in Kubura Well was estimated by dilution sampling to be 48 (95% CI 45 and 52) and 43 (95% CI 38 and 50) fish in June and July 1993, respectively (Humphreys<sup>2</sup>).

### Food and stable isotope ratio analysis

Young (1986) reported that, in captivity, *M. veritas* ate mosquito larvae, live brine shrimps, and that eventually they would take frozen brine shrimps and would take prey up to one-third of their body length. He observed that *M. veritas* often missed live prey on the surface film but rarely missed them when live prey were in the water column; they took live prey by a sudden gulp while nearly stationary and with the fins flared. When offered food they frequently ignored it and allowed tubificid worms to move over them without overt response even though they eventually ate them. Allen (1989) considered *M. veritas* to feed opportunistically upon detritus, algae and whatever organisms, including insects, that accidentally fell into the water. Examination of the intestinal contents of existing collections showed that the diet of *M. veritas* included terrestrial (79%) and aquatic species and that amongst the aquatic species both stygobitic (10%) and epigeic species were ingested (Humphreys & Feinberg 1995). The predominant prey of terrestrial origin consumed by *M. veritas* differed between the east and west coast of the peninsula, reflecting the land use. Exotic cockroaches (*Periplaneta*) and native isopods (*Buddelundia*) predominated in the diet of fish, respectively, inhabiting the settled east coast and the largely unpopulated west coast (Humphreys & Feinberg 1995). Hence, the fish appear to feed opportunistically on terrestrial invertebrates accidentally introduced into the aquatic system (Humphreys & Feinberg 1995). Recent observations (1999) of *Buddelundia* foraging underwater in Kubura Well means that their presence in water is not necessarily accidental. *M. veritas* also includes in its diet aquatic larvae of aerial species (chironomid and caddis larvae) as well as specialized members of the stygofauna (*Stygiocaris* sp.) (Humphreys & Feinberg 1995). The bigger west coast fish were associated with bigger food items (isopods). However, as terrestrial Crustacea generally have an energy density only about 70% that of terrestrial insects (from Cummins & Wuycheck 1971), then this apparent advantage in food size may not confer an energetic advantage.

The gut contents examined by Humphreys & Feinberg (1995) were predominantly from large individuals all of which had been sampled from wells or open caves where there was direct open access between the ground surface and the groundwater and hence where epigeic prey species would be present. Throughout most (>99.99%) of the inferred distribution of



this obligate groundwater community (stygofauna) there are no known openings to the surface. Extensive sampling down boreholes has shown that stygo-fauna, including *M. veritas*, is present in such areas, from where there is a total absence of epigeal species (Humphreys & Feinberg 1995). Hence, in general, the fish are probably dependent on the stygofauna for food rather than on epigeal species accidentally in the water.

The fish share their habitat with a diverse stygo-fauna, many of which were not represented in their gut contents (Humphreys & Feinberg 1995), probably owing to the specimens having been sampled at sites open to the surface where stygofauna would have been scarce and where prey of epigeal origin was abundant. The smallest taxa, such as copepods and ostracods, may lie outside the size range of prey items, which for *M. veritas* is known to include the length range of 2.8–14 mm, and for *O. candidum* 3 mm (*Halosbaena*) to an estimated 8 mm (Odonata). Melitid amphipods are abundant and sympatric with *M. veritas* but they were not identified from the gut contents despite being within the known size range of prey (Humphreys & Feinberg 1995).

The prey identified is consistent with the behaviour of the fish. *M. veritas* moves widely through the water column, often hovering in mid- to surface waters where prey with hydrophobic integuments, such as cockroaches, would be encountered when they fell into the water. In contrast, *O. candidum* inhabits the surface of, and burrows into, the flocculent faecal ooze characteristic of crustacean-rich stygal habitats, and all the prey items identified were bottom dwellers or, in the case of the isopod, would sink to the bottom when it fell into the water.

To further examine the food of *M. veritas* stable isotope ratio analysis was conducted on elements of the stygofauna and surrounding habitats (methods in Humphreys 1999b). The locations of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and the trajectory of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  coordinates between trophic levels provides information on the trophic level of the species as well as the origin of the food source for each trophic level (detailed in Humphreys 1999b).

The stable isotope signature (Figure 4) provides strong evidence that *M. veritas* is a predator and does not rely to any extent on detritus or algae as a food source as suggested by Allen (1989). Furthermore, the  $\delta^{13}\text{C}$  ratios suggest that they depend on the energy derived from plants using the C4 photosynthetic pathway, a mixture of C3 and C4 plants, and from chemoautotrophic energy fixation by

sulphur bacteria via stygal shrimps (Atyidae) scraping the biofilm in the anoxic/oxic ecotone in the anchialine sinkhole at Bundera (details in Humphreys 1999b).

#### *Size, growth, breeding and condition*

*Milyeringa veritas* ranged from 5 to 57 mm standard length (SL) and those collected from the east coast were bigger in every respect from those on the west coast but the meristic variables do not differ between areas (Humphreys 1994, figure 13). For example, the mean SL of fish from the west coast was 32.8 mm (7.9 = SD; 125 = sample size) compared with a mean elsewhere of 24.6 mm (10.4, 23,  $F_{31,146} = 18.67$ ,  $p < 0.001$ ).

*Milyeringa veritas* isolated in a cave pool for 42 days grew in length at a rate of 0.6 mm per month (95% limits 0.2–1.0 mm) but during this time lost weight indicating that their conditions may not have been optimal.<sup>2</sup> These sparse data on growth rate are consistent with the apparent cohort size indicated from length frequency distributions and suggest that the larger fish are 3+ years old (Figure 5).

The only data available on seasonal change in condition in *M. veritas* are derived from museum specimens. The condition index differs significantly through the year being higher in August and September than in other months (Table 2). There are inherent problems with the use of such material, including preservation artifacts and poor distribution in the samples of the size-classes and seasons. However, the results are of interest as small individuals (less than 18 mm SL, cf. maximum 57 mm SL) have been observed in December–March in several years (K. Cameron personal communication) suggesting that there may be a distinct breeding season. The style of breeding in *M. veritas* is unknown. The smallest individuals caught are 4.5 mm SL.

Annual, even seasonal breeding cycles may be unexpected in tropical cave animals. What could be the cue to seasonal breeding in *M. veritas* suggested by the 'cohorts' in Figure 5 and the apparent periodic appearance of small individuals? The growth data and size class frequency analyses were derived from *M. veritas* sampled from caves and open pastoral wells and so they are not dependent on the stygal ecosystem as they gain most of their food directly from animals inhabiting terrestrial habitats (Humphreys & Feinberg 1995). Such *M. veritas* populations, closely tied to surface openings, may be expected to respond differently to those populations inhabiting groundwaters more remote from surface openings and dependent

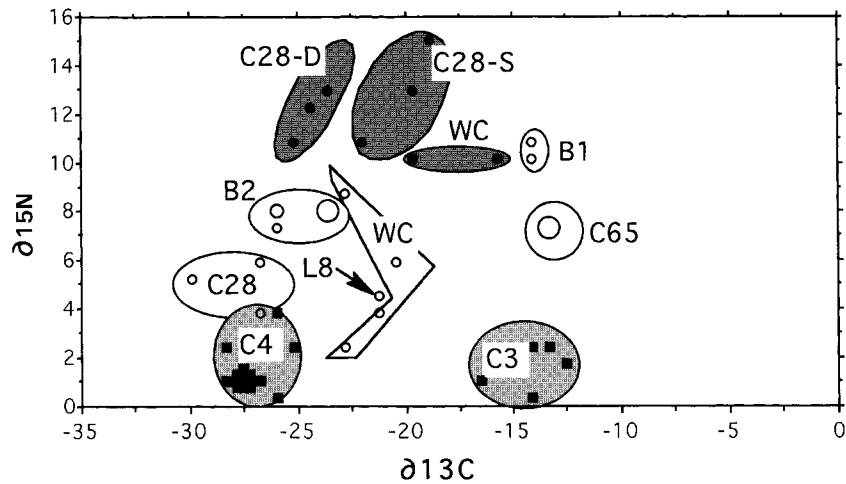


Figure 4. Stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of *Milyeringa veritas* (dark shading: C28-D and C28-S = deep and shallow samples, respectively, from Bundera Sinkhole, west coast of the Cape Range peninsula (Humphreys 1999b); WC = east coast of the Cape Range peninsula and some elements of the associated system. Light shading are plants with C3 and C4 photosynthetic pathways. Unshaded = *Stygiocaris stylifera* from various sites (C28 = Bundera Sinkhole; WC = east coast of the Cape Range peninsula; B2, L8 and C65 = anode protection wells on Barrow Island; B1 = anchialine cave on Barrow Island). The location of the *M. veritas* data appears to reflect the widely varying stable isotope signature of the largest prey, the atyid shrimp *Stygiocaris*. It has been argued from these and other data that in places the atyids depend on chemoautotrophic energy fixed by sulphur bacteria associated with the oxic/anoxic interface (Humphreys 1999b). Data for *Stygiocaris* and *M. veritas* from location WC are from Davies, P.M. 1996. Stable isotope analyses of the food-webs associated with stygofauna of Exmouth borefield. A report to the Water Corporation Appendix 1, 13 pp. In: Supplementary Investigations of the Effects of Public Water Supply Abstraction on the Stygofauna and Aquifer of the Cape Range, Water Corporation, Perth.

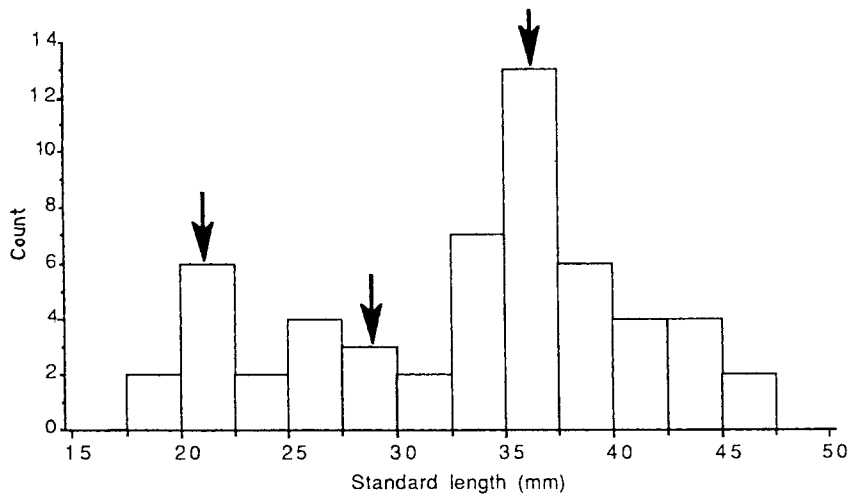


Figure 5. The pooled size class distribution of *Milyeringa veritas* from Kubura Well in 1993. Arrows denote the projected movement of the lower 'cohort' over successive years as estimated from the measured growth rate (see text) in Kubura Well.

Table 2. Condition index (standard length (mm)/  $3\sqrt{\text{weight (g)}}$ ) for *Milyeringa veritas* determined from museum specimens.

Month	Mean	SE	N
May	38.2a	0.28	46
June	37.0a	0.36	19
July	38.3a	0.46	31
August	39.8b	0.49	24
September	39.8b	0.81	18
October	38.1	0.60	5

The means differ significantly ( $F_{5,137} = 4.373$ ,  $p = 0.001$ ; October samples excluded owing to small sample size). Rows with common letters are not significantly different by the Fisher PLSD test. The samples were fixed in formaldehyde and represent combined samples from several years of collecting in the 1960s.

on stygal prey species. Cave and well populations of *M. veritas* (and other stygofauna, such as *Stygocaris* spp.) may be expected to respond closely to changes in food resources rather than to variation in the stygal community itself. The episodic heavy rainfall associated with summer cyclones may serve to wash copious food into the caves, or else the humid caves may serve as a refuge for terrestrial invertebrates during the very dry and hot (frequently  $> 40^{\circ}\text{C}$ ) summer period. Clearly, detailed work on the fish is required.

### Morphometrics

The morphometric development of cave fish has largely been neglected other than the general observations that they often have reduced body size and larger head and fins (Poulson 1964) and that this may be a 'neotenic' trend (Weber et al. 1998a). However, no thorough examination of the heterochrony (McNamara 1990) of cave fish has been undertaken with respect of their lineages or considered generally as a stygomorphy (cf. troglomorphy: Christiansen 1962, Humphreys 2000c).

A synopsis of the morphological trends in *M. veritas* is presented here (Figure 6, Table 3) for comparison with other species of cave fish. The general trends apparent in these data are that as the fish grow they become more bulky, the tail becomes shorter, the body becomes deeper and narrower and the buccal region shorter. How this development pattern relates to habits is unknown.

### Genetics

Seven populations of *M. veritas*, from throughout its distribution on the Cape Range peninsula, were subjected to a comprehensive allozyme analysis at 43 loci (Adams & Humphreys 1993), a synopsis of their findings is presented here. Of the 43 loci scored, 28% of loci were polymorphic ( $P$ ) and the average level of heterozygosity per population ( $H$ ) was  $0.028 \pm 0.013$  ( $n = 7$ ). Compared with marine fish, generally, *M. veritas* exhibits low  $H$  but high  $P$ : for 169 populations of marine fish, after removal of studies involving few individuals ( $< 20$ ), Planes (1998) found  $H = 0.058 \pm 0.037$  and  $P = 21.37 \pm 14.09\%$ . Planes (1998) found no effect of taxonomic placement in marine fish on the magnitude of  $H$  or  $P$ . Low values of  $H$  have sometimes been associated with small populations (Soulé 1976, Nevo et al. 1984), stable environments (Levins 1969, Van Valen 1965) or habitat specialization (Nevo 1978) associated with *M. veritas*. However, as no comparable allozyme data are available for other eleotrids, such comparisons are premature.

The average Nei's  $D$  for all populations was 0.04, and an average of 2.3%, with a maximum of 7% of loci had fixed differences. The mean  $F_{ST}$  between populations was 0.457 (95% CI = 0.211–0.599) while the mean FIS between populations was 0.208 (95% CI = 0.071–0.300). Whilst some fixed differences were encountered, the overall levels of genetic differentiation between populations and the geographic pattern of allele distribution were both consistent with the presence of a single biological species (Figure 7). However, analysis of the F-statistics demonstrates the presence of significant population substructuring in this species. The average gene flow per generation ( $Nm$ ) can be determined from the conditional average frequency,  $F_{ST}$  (Slatkin 1981). In an island population model  $Nm$  represents the average number of individuals exchanged between populations in each generation and populations do not diverge unless  $Nm < 1$  (Wright 1931). Generally, if  $F_{ST} > 0.2$  ( $Nm < 1$ ) considerable divergence will occur between populations, and with values of  $F_{ST} > 0.33$  ( $Nm < 0.5$ ) populations will be essentially unconnected genetically (Trexler 1988, Slatkin & Barton 1989). Hence, the population of *M. veritas* is not genetically uniform and there is evidence of restricted gene flow on the peninsula (Humphreys & Adams 1991, Adams & Humphreys 1993), as is also the case in the sympatric shrimp *Stygocaris stylifera*. As some populations of

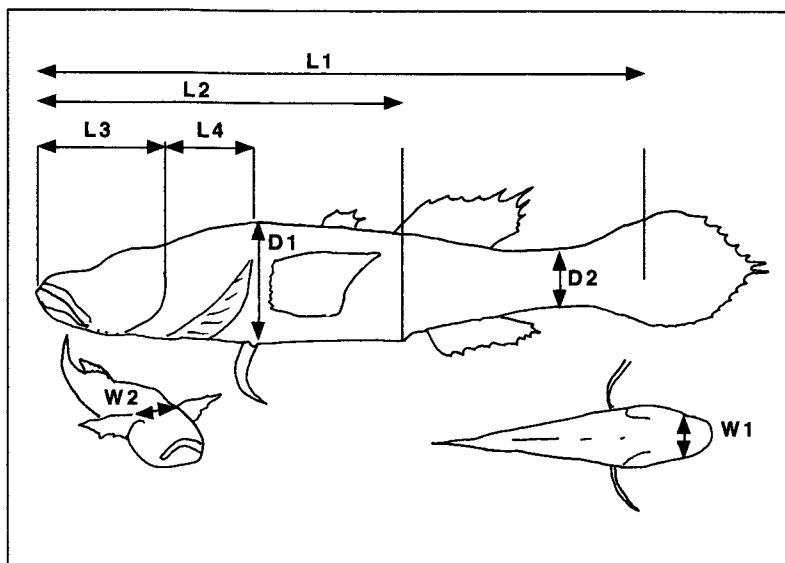


Figure 6. Morphometric measurements on *Milyeringa veritas* (see Table 3) from specimens in the Western Australian Museum.

Table 3. Morphometric relationships: regressions of linear measurements and weight<sup>-3</sup> of *Milyeringa veritas* ( $N = 148$ ) summarized in the matrix of slope (upper right) and  $r^2$  (lower left) of regressions of log  $X$  (vertical) on log  $Y$  (horizontal) of variables L1 = standard length (size range examined was 5–57 mm); L2 = snout vent length; L3 = length 3 (see Figure 6); L4 = length 4; W1 = width 1; W2 = width 2; D1 = depth 1; D2 = depth 2. The slope is shown only when the 95% confidence intervals for the slope do not include a slope of 1.0.

	Wt	L1	L2	L3	L4	D1	D2	W1	W2	L1–L2
Wt	—	0.95	—	0.90	—	—	1.04	—	0.96	0.93
L1	0.98	—	—	0.94	1.06	1.05	1.08	—	—	—
L2	0.97	0.98	—	0.91	—	—	—	—	—	0.92
L3	0.96	0.97	0.95	—	1.09	1.10	1.13	—	1.05	—
L4	0.97	0.95	0.95	0.91	—	0.94	—	0.91	0.91	0.87
D1	0.98	0.93	0.92	0.92	0.89	—	—	0.94	0.91	0.87
D2	0.98	0.94	0.93	0.93	0.90	0.92	—	0.90	0.89	0.85
W1	0.94	0.87	0.87	0.86	0.84	0.89	0.88	—	0.90	0.85
W2	0.96	0.96	0.95	0.94	0.94	0.93	0.94	0.90	—	0.93
L1–L2	0.90	0.94	0.85	0.89	0.85	0.86	0.86	0.79	0.93	—

*M. veritas* are genetically isolated from others, this may suggest that more than one biological species is present.

The genetic relationships amongst populations are displayed visually in Figure 7. Two major clusters are evident and these reflect a general 'western' versus 'eastern' geographic split. Populations C-27 and C-361 from the east coast of Cape Range peninsula are invariant at all loci examined, and display 5–7% fixed differences when compared to all but one of the

populations on the west coast. The exception is the geographically intermediate well C-273 at the northern end of the peninsula, which is genetically intermediate between the two groupings. This within-species geographic trend broadly mimics the distribution of the two species of shrimp, which overlap in C-24, C-25, and C-273 (Humphreys & Adams 1991, Adams & Humphreys 1993). The gudgeons are the only group examined in which the F-statistics suggest there may not be random mating occurring within subpopulations

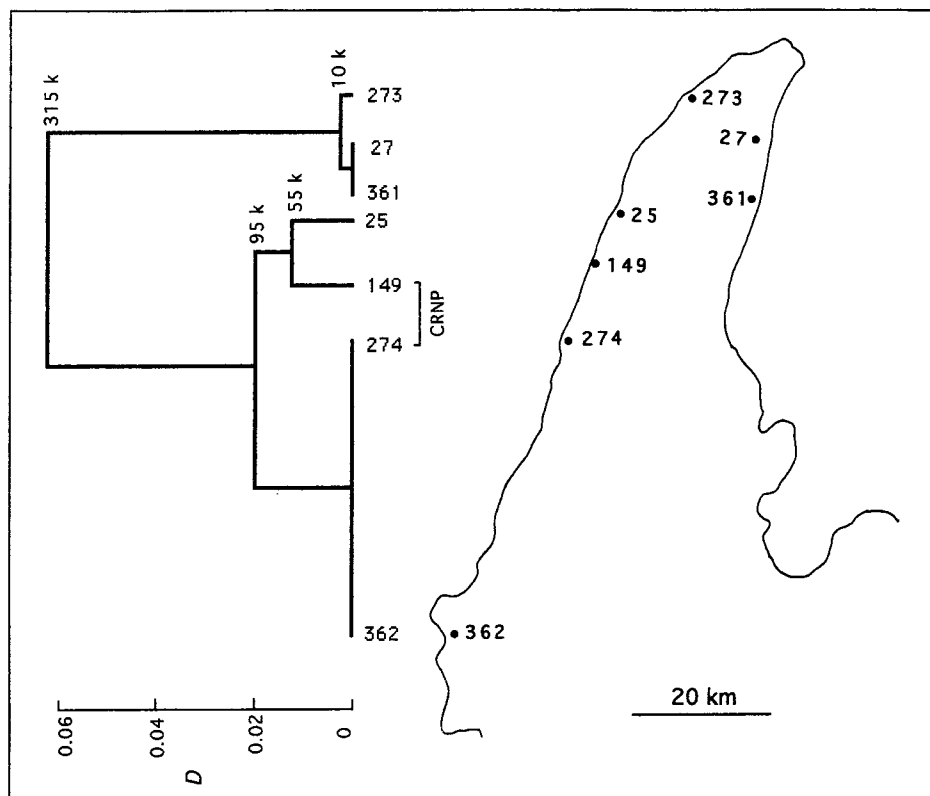


Figure 7. Dendrogram depicting the genetic relationship (Nei's  $D$ ) amongst the seven populations of *Milyeringa veritas* (data from Adams & Humphreys 1993) plotted alongside a map depicting the position of the populations sampled. The numbers on the dendrogram are the molecular clock ages (thousand years) derived from Nei's relationship (Nei 1987) as discussed in the text. The numbers alongside the dendrogram and on the map denote the site numbers as recorded in the karst index for Cape Range. CRNP denotes the two populations sampled that are included within Cape Range National Park. Top of the map is north.

but it is not possible to explore the significance of this result any further without additional sampling.

Many cave organisms appear to conform to the molecular clock (Sbordoni et al. 1990) proposed by Nei (1987) in which one unit of Nei's  $D$  is equivalent to 5 million years of divergence between populations. Using this relationship, the estimated divergence times between the *M. veritas* populations (Figure 7) are concordant with major periods of low sea level, up to 130 m below that now present. Three of the last four extreme sea level minima occurred at about 95 000, 55 000 and 20 000 years ago (Wyrwoll et al. 1993), the estimated times of the three most recent divergences between the *M. veritas* populations. There was another major sea level minimum at ca. 315 000 years ago (Hays et al. 1976), coinciding with the estimated time of the initial divergence between the *M. veritas* populations.

These data are consistent with the hypothesis that the regional genetic structure between *M. veritas* populations was determined by vicariance associated with eustatic events.

### Conservation status

The remote location of these cave fish and their subterranean habitat restricts observation in life and hence very little is known about the biology of either sympatric species. Nonetheless, *M. veritas* and *O. candidum* have, respectively, been classified as rare and recommended for total protection, and vulnerable (Michaelis 1985). Both fish species (as well as some stygal crustacean species) have been listed as endangered under Schedule One of the Wildlife

Protection Act of Western Australia owing to their small geographic distribution, low populations and vulnerability of their habitat. Bundera Sinkhole and Camerons Cave, sites inhabited by *M. veritas*, are classified as endangered communities by the Western Australian wildlife authorities. The conservation status of these species is discussed by Romero & Vanselow (2000a,b).

While there has been a significant extension of the range of *M. veritas* to Barrow Island (Humphreys 1999c), the inclusive known range of the two species on the Cape Range peninsula has not increased since 1991 (Humphreys & Adams 1991). The number of sites from which *M. veritas* has been recorded has increased steadily over time, from two sites in 1962 to 25 sites in 1999 (Humphreys 1999c). These additional sites have markedly increased the known range of habitats and water types inhabited by the species. However, 19 of these sites are insecure or have been lost – six of these access sites have been lost in the intervening period due to infilling, drying or siltation of the sites, and a further 10 sites are close to planned developments or are within an urban area, and three are within a military bombing range reserve. Only six sites are within Cape Range National Park where infrastructure developments are also planned. *M. veritas* is certainly more widespread in the karst than indicated by the point samples. Despite this, the loss of access sites is crucially important as they provide the only windows into the karst system to enable the biological study and monitoring of cave fish populations and their associated ecosystems.

Owing to the genetic diversity and restricted gene flow in *M. veritas* on the peninsula (Humphreys & Adams 1991, Adams & Humphreys 1993), the presence of *M. veritas* within Cape Range National Park does not in itself provide an adequate representation of the genetic diversity of the species (Figure 7), nor does it adequately represent their habitat diversity.

The stygal community on the Cape Range peninsula is unusual in having two species of sympatric cave fish within a rich community of stygal Crustacea, many belonging to relict groups. As the fish and crustaceans are part of the same food web (Figure 4), the broad conservation implications are clear—the stygofauna is a functional community, changes to part of which may affect the remainder. Such relatively simple ecosystems are potentially vulnerable to disruption by ‘top-down’ effects (e.g. removal of *M. veritas* as the top predator), and *M. veritas* susceptible to ‘bottom-up’ effects (e.g. changes to biofilms by nutrients), factors, amongst

others, discussed by Boulton (2000) for subsurface systems.

## Prospects

Although information on *M. veritas* is sparse, and of variable quality, it is clear that this species inhabits waters with an extraordinary physico-chemical and biological variety. There are clear interruptions to the gene flow within the Cape Range peninsula that may reflect earlier eustatic events.

Few sites have been examined in respect of the allozyme variation in *M. veritas* and a more complete sampling may provide a more complex picture of the variation between local populations, and of the vicariance hypothesized to be associated with eustatic events. In addition, the non-random mating apparent from the allozyme data suggests that detailed population genetics using molecular markers would provide insights into the population processes of the cave fish.

Individual long term marking is needed to provide the basic population data required intrinsically and for possible management needs, such as growth rate, dispersal, dispersion, population size and structure, and breeding. This would also facilitate examination of the physico-chemical environment variation for a given population, which, with their physiological tolerance or ability to conform, are at present unknown.

The degree of ocular regression is unknown, as is the degree and nature of the stygomorphies. Finally, the affinities of the species within the Eleotridae are unknown, the resolution of which would stimulate research on the Australian cave fishes. It is now known that the populations of *M. veritas* are of sufficient size to sustain a carefully planned major research effort. Despite the general remoteness of the area, the populations occur at sufficiently accessible locations to facilitate sampling.

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