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A new species of the blind cave gudgeon *Milyeringa* (Pisces: Gobioidi, Eleotridae) from Barrow Island, Western Australia, with a redescription of *M. veritas* Whitley

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Abstract

A new species of the eyeless eleotrid genus *Milyeringa* is described from wells sunk on Barrow Island, Western Australia. *Milyeringa justitia* n. sp. is the third species of the genus to be named. Morphological data and cytochrome *c* oxidase subunit I (COI) DNA sequence data from a wide sample of localities at which the genus occurs was used to evaluate relationships and species limits. *Milyeringa veritas* is redescribed, and *M. brooksi* is synonymised with *M. veritas*. The unique form and ecology of these fishes, plus the threats to their survival, warrants immediate and continuing attention in management.

Key words: Eleotridae, *Milyeringa*, new species, caves, stygofauna, Western Australia

Introduction

The cave gudgeon, *Milyeringa veritas* Whitley, 1945, has been known as the only cave-dwelling gobioid in Australia. It had been reported only from anchialine caves and wells at the base of and foothills of the Cape Range peninsula, Western Australia and from Barrow Island, some 180 km distant (Adams & Humphreys 1993; Humphreys 2001a; Chakrabarty 2010) (Figs 1, 2). Whitley (1945) was so impressed by the species' eyelessness and reduced dorsal and ventral fins that he placed it in its own family, Milyeringidae, which was subsequently synonymised with the Eleotridae by Mees (1962). Later, Hoese and Gill (1993) placed *Milyeringa* in the eleotrid subfamily Butinae. Thacker and Hardman (2005) placed *Milyeringa* in the Odontobutidae based on an analysis of four mitochondrial genes, as did Thacker (2009), using the same four genes plus additional taxa. However, in the latter study *Milyeringa* flipped into a polytomy with *Rhyacichthys* and the odontobutids *Odontobutis* and *Perccottus* (Thacker 2009: Fig. 1). In contrast, Mooi and Gill (2008), using 55 morphological characters, presented a consensus tree (of six equally parsimonious trees) in which *Milyeringa* forms a polytomy with the butine *Hannoichthys* and the rest of the gobioids above the odontobutids and rhyacichthyids.

Chakrabarty (2010) recommended retaining the family name Milyeringidae for *Milyeringa* "... to highlight its distinct position within the Gobiiformes and for its extreme ecological specializations". Most recently, *Milyeringa* has been presented as sister-group to the Eleotridae in a clade with *Typhleotris*, the very similar blind cave gudgeon from Madagascar (Chakrabarty *et al.* 2012). None of the analyses to date have included sequence data from nuclear genes. Clearly there remains considerable work to be carried out on gobioid relationships and to reconcile the differing morphological and molecular analyses and approaches (see Mooi & Gill 2010). We have retained *Milyeringa* in Eleotridae until further evidence is acquired.



FIGURE 1. Looking south along Cape Range coastal plain, Western Australia. Photograph by W.F. Humphreys.

Much has been written about *Milyeringa veritas* as a blind cave fish and its status as a threatened species (Adams & Humphreys 1993; Humphreys 2001a,b; Humphreys *et al.* 2006; Romero & Vaneslow 2000). However, its morphological features have never been fully described apart from its general appearance and changes in form with growth (e.g. Mees 1962; Allen 1982; Whitley 1945; Humphreys 2001a).

Humphreys and Adams (1991) and Adams and Humphreys (1993) carried out the first genetic work on the different populations of *Milyeringa* along the Cape Range (including those subsequently named as *M. brooksi* Chakrabarty, 2010), using allozyme electrophoresis. They considered that the allele distribution, across a comprehensive sample of localities, was consistent with that of a single biological species. Despite this finding, a second species of *Milyeringa* from a narrow distribution in the south-west of Cape Range, *M. brooksi*, was recently described on the basis of purported mitochondrial haplotype divergences and morphological differences from *M. veritas* (see Chakrabarty 2010). The lack of allozyme-data support for the recognition of two species on the Cape Range peninsula warranted clarification of the taxonomic status of *M. brooksi*.

Specimens from Barrow Island were not available for earlier studies, but when one eventually became available, it became clear from preliminary allozyme results that it was very divergent from mainland *Milyeringa* (M. Adams, unpubl. data). Additional specimens of the Barrow Island population of *Milyeringa* were recently obtained for morphological study and DNA analysis. We found that the population from Barrow Island reported by Humphreys (2001b) represented a distinctive species, described here as *M. justitia* n. sp.

Methods

Morphology. Measurements were taken using electronic callipers and dissecting microscope. Counts and methods generally follow Hubbs and Lagler (1970), except as indicated below. Transverse scale counts backward (TRB) are taken by counting the number of scale rows from just before the anal fin origin diagonally upward and back toward the second dorsal fin base. Head length is taken to the upper attachment of the opercular membrane. As the genus has no eyes, two additional head proportions were taken as follows: one from the snout tip to the upper edge of the preopercular margin (snout to POP) and one from the upper edge of the preopercle to the upper margin of the

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FIGURE 2. Bundera sinkhole, Cape Range; anchialine cave containing a population of *Milyeringa veritas* throughout its 32 m depth and a rich anchialine crustacean fauna including Remipedia. Photograph by W.F. Humphreys.

opercle (opercle length). The total segmented caudal ray pattern (e.g. 9/8) is the number of segmented caudal rays on either side of the gap between the two parts of the hypural plate (hypurals 3–4 and 1–2). Vertebral counts and other osteological information were obtained by X-ray (*M. veritas*) and X-ray microtomography (CT scanning) (*M. justitia n. sp.* and *M. veritas*). Pterygiophore formula follows Birdsong *et al.* (1988). Museum acronyms are as in Leviton *et al.* (1985).

Mitochondrial Genotyping. The sampling for mainland *Milyeringa* was based on the population sub-structure reported by Adams and Humphreys (1993), in order to gain a better understanding of haplotype distribution and its systematic implications. A fragment (600 bp) of the cytochrome *c* oxidase subunit I (COI) gene sequence was sequenced from representative samples of all available mainland (Cape Range) *Milyeringa* populations. Tissue samples from allozyme studies (Humphreys & Adams 1991; Adams & Humphreys 1993) were sourced from the Australian Biological Tissue Collection (ABTC), held at the South Australian Museum. Fin clip samples of *M. justitia n. sp.* were obtained from the Western Australian Museum. Details of all samples are listed in Table 1 along with the GenBank accession numbers of sequences.

Total DNA was extracted from allozyme homogenates or alcohol preserved tissue (muscle or fin) using a DNAzol (Invitrogen) tissue extraction kit following the manufacturer's protocol. PCR primers (GOBYL6468 & GOBYH7127) and protocols were those of Thacker and Hardman (2005) as used by Chakrabarty (2010). Products were purified and sequenced in both directions at the Australian Genome Research Facility (AGRF, Brisbane). Sequences were edited using Seqed (ABI) and manually aligned in Se-Al v2.0a11 (Rambaut 1996).

TABLE 1. Tissue (ABTC = Australian Biological Tissue Collection), field, voucher and GenBank accession numbers and locality information for genotyped individuals of *Milyeringa*. Well numbers as per Adams and Humphreys (1993). Asterisks indicate samples of *M. brooksi* COI haplotype, while # indicates the sample of *M. justitia n. sp.* (WAM P.33135-001, paratype).

ABTC No.	Sample No.	Voucher Specimen	Cave/Well	Genbank Number
NA	BES 15151#	WAM P.33135-001	Anode Well Q4	KC142150
ABTC2289	C25A*	No Voucher	C-25	-
ABTC2289	C25B	No Voucher	C-25	-
ABTC2289	C25C	No Voucher	C-25	-
ABTC2289	C25E*	No Voucher	C-25	-
ABTC2290	W1A	No Voucher	C-273	-
ABTC2290	W1B	No Voucher	C-273	-
ABTC2290	W1F	No Voucher	C-273	-
ABTC2290	W1G	No Voucher	C-273	-
ABTC2291	W2A*	No Voucher	C-362	-
ABTC2291	W2B*	No Voucher	C-362	-
ABTC2292	W3A*	No Voucher	C-274	-
ABTC2292	W3B*	No Voucher	C-274	-
ABTC2293	W4B	No Voucher	C-27	-
ABTC2293	W4C	No Voucher	C-27	-
ABTC2294	W5A	No Voucher	C-361	-
ABTC2294	W5B	No Voucher	C-361	-
ABTC2294	W5C	No Voucher	C-361	-
ABTC2288	C149A*	No Voucher	C-149	-
ABTC2288	C149B*	No Voucher	C-149	-
ABTC2288	C149C*	No Voucher	C-149	-
ABTC2288	C149D*	No Voucher	C-149	-
ABTC2288	C149E*	No Voucher	C-149	-

Outgroup taxa selected were *Perccottus glenii* and *Odontobutis potamophila* from GenBank, as per Chakrabarty (2010). *Milyeringa* COI sequences from that study (631 bp, but reported as 1107 bp), available from GenBank, were incorporated into the analysis as were those of *Typhleotris* species (Chakrabarty *et al.* 2012). The combined alignment was used to generate a Neighbor-Joining (NJ) tree (Kimura 2-parameter) in PAUP* 4.0 (Swofford 2001).

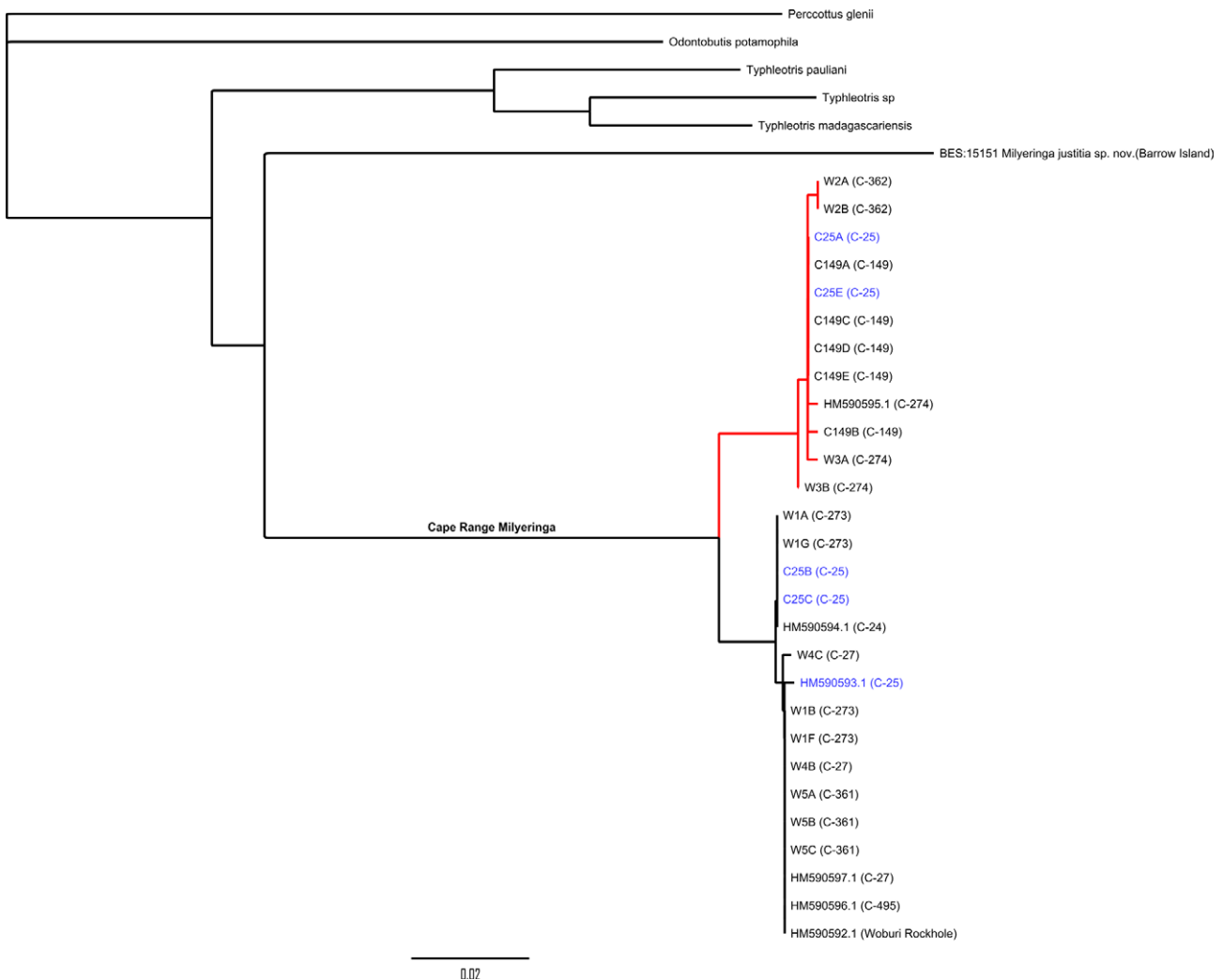


FIGURE 3. *Milyeringa* COI neighbor-joining tree. Collection localities of samples are in parentheses following the sample number. Red indicates the *M. brooksi* haplotype clade and blue indicates those individuals collected from Kudumurra Well (C-25).

Systematics

Milyeringa Whitley, 1945

Diagnosis. An eyeless eleotrid with first dorsal fin reduced (spines II–IV) to absent, segmented second dorsal fin rays 6–8 with no spine present; anal fin rays I,6–8 or 6–7; pectoral fin rays 11–13, all unbranched; pelvic fin rays I,3–4, all unbranched; segmented caudal fin rays 13–17, all unbranched and central rays sometimes elongate and filamentous; body scales cycloid, covering body in one species and almost absent in another; sensory canals and pores on head (and body) completely absent; sensory papillae on head in longitudinal pattern and on body in reduced vertical rows, papillae with very thin pointed flaps present (easily lost); head moderately long, becoming wide and depressed in large specimens; body fairly slender, compressed; jaws with few rows of small pointed teeth; body pigment absent but for few scattered fine brownish melanophores on top of head, fin membranes transparent to pale translucent whitish; vertebrae 7–10+13–15 (20–25 in total), 1–2 pre-anal pterygiophores, one epural; last

haemal spine usually split or forked (with thin sheet of bone joining forks). Known so far only from caves and wells in the Cape Range peninsula and Barrow Island, Western Australia.

Molecular genetic data. The recovered COI gene tree is shown in Fig. 3. The tree indicates a clear dichotomy within the genus as presently defined, with the Barrow Island *Milyeringa* COI sequence differing from that of mainland *Milyeringa* by 20%. This is equivalent to the divergence of each from *Typhleotris*, only slightly less than that from the odontobutid outgroup taxa, providing evidence of two very different species and strongly implying that the split between the two Western Australian species is ancient. Our unpublished preliminary molecular data indicate that this split is indeed ancient, which may have systematic implications. We are investigating this further, in light of Chakrabarty *et al.* (2012), who proposed that *Milyeringa* and the Madagascar cave gudgeon *Typhleotris* are sister-lineages.

It is not possible to assess the level of genetic variation within *M. justitia* n. sp. on Barrow Island at present, given the very limited sampling. A second individual from Anode well Q4 had an identical COI haplotype: however, additional sampling and analysis may yet reveal diversity and population sub-structuring within a very restricted region, as for *M. veritas*. Further assessment of the intraspecific variation is crucial, for clarification of the conservation status and management of this Barrow Island endemic.

Milyeringa justitia, new species Larson and Foster

Barrow cave gudgeon

Diagnosis. A *Milyeringa* with first dorsal fin absent (remnant of single pterygiophore present in CT-scanned specimen), 6 segmented second dorsal fin rays; 6–7 segmented anal fin rays; pelvic fin rays I,3; 13–15 caudal fin rays; caudal and pectoral fin rays all unbranched; an almost naked body with greatly reduced scalation (single row of few cycloid scales present), head short with reduced numbers of rows of sensory papillae on the head and body; vertebrae 7–9+13–15 (22 in total), one pre-anal pterygiophore, one epural, last haemal spine broad, split, with sheet of bone joining forks. Restricted to subterranean aquifers accessed by wells sunk on Barrow Island, Western Australia.

Material examined. All from Barrow Island, Western Australia. HOLOTYPE: WAM P.33166-001, 19.5 mm SL male, old water supply well, L8, 20° 49' 2.31" S 115° 23' 41.98" E, BES 9794, coll. G. Humphreys and J. Alexander, 25 November 2009. PARATYPES: WAM P.33167-001, 19 mm SL male, old water supply well, L8, 20° 49' 2.31" S 115° 23' 41.98" E, BES 9795, coll. G. Humphreys and J. Alexander, 25 November 2009; WAM P.33137-001, 16 mm SL female, Anode well Q4, 20° 59' S 115° 24' E, BES 15152, coll. W. Hayes and J. Sherborne, 12 August 2009; WAM P.33135-001, 16 mm SL, sex uncertain, Anode well Q4, 20° 47' 28" S 115° 23' 57" E, BES 15151, coll. W. Hayes and J. Sherborne, 12 August 2009; WAM P.33169-001, 23 mm SL female, Anode well P2, 5.2 km W of terminal tanks, 20° 46' 40.07" S 115° 24' 41.77" E, BES 15354 (15152 in original paper), coll. W. Hayes and J. Sherborne, 6 December 2009.

Non-type material: WAM P.33165-001, anterior half of body remaining, mid-point of old water supply well, L8, Barrow Island, 20° 49' 02.2" S 115° 23' 42.2" E, BES 6959, coll. K. Hallett, 13 January 1999.

Description. Based on five specimens, 16–23 mm SL. An asterisk indicates counts and measurements of holotype (Fig. 4).

First dorsal fin absent; second dorsal rays 6*; anal rays 6*–7; pectoral rays 12*–13; pelvic rays I,3; segmented caudal rays 13–15*, in 7/6 pattern in four, 8/7 in holotype; 7/6 (in 3), 7/7 (1), 8/6* (1), 9/9 (1) segmented caudal fin rays, all rays unbranched; lateral scale count 2–8*, in single row; vertebrae 7+15 (1), 9+13 (1); single large epural; one anal pterygiophore anterior to first haemal spine; last haemal spine broadened and forked (poorly ossified).

Body rather short, compressed, more compressed posteriorly; body depth at anal fin origin 15.2–18.4% of SL. Caudal peduncle moderate, length 19.5–21.3% of SL. Caudal peduncle depth 9.2–10.6% of SL. Head slightly depressed, rounded to rather square in cross-section, wider than deep at preopercular margin, head length 36.9–40.0% of SL; head depth at posterior preopercular margin 48.4–54.8% of HL; width at posterior preopercular margin 54.2–64.8% of HL. Mouth moderately large, terminal and oblique, chin tip anteriormost, jaws forming an angle of about 35° with body axis. Upper jaw length 43.8–47.5% of HL; inner margin of lips smooth; lower lip fused to chin anteriorly, side of lip free; chin flat and smooth. Anterior naris in very short tube at edge of upper lip, posterior naris with slightly narrower opening, nares joined by thin fleshy tube over nasal rosette; nares may be

reduced to two low-rimmed pores joined by short tube over nasal rosette. Eyes absent. “Snout” area between tip of jaws and rear preopercular margin broad and concave, with hump behind maxillary symphysis, snout forming rounded square when viewed from above, length of snout tip to upper edge of the preopercular margin 68.8–76.7% of HL. Opercle length (upper edge of preopercle to upper margin of opercle) 25.4–31.5% of HL. Preopercular margin bony, smooth and flat. Gill opening very wide, extending to just anterior to or just below rictus. Tongue large, tip gently rounded to slightly pointed. Teeth in both jaws small, evenly sized, conical and pointed; in two rows; largest teeth at front of upper jaw and along side of lower jaw. Headpores absent. Sensory papillae on head in reduced longitudinal pattern (Fig. 5); many papillae with thin narrow flap each (often missing due to their fragility). Sensory papillae on body reduced, with single uneven row of papillae along mid-side, a vertical row of few papillae anterior to hypural crease and another row on base of caudal fin just behind hypural crease; papillae on body rarely with thin narrow flap (probably due to damage) (Fig. 6).

TABLE 2. Measurements of all type specimens of *Milyeringa justitia* n. sp., expressed as percentage of standard length (SL) or head length (HL). Missing data is due to specimen being damaged or distorted (POP = preopercular posterior margin).

	Holotype WAM P.33166-001	Paratype WAM P.33135-001	Paratype WAM P.33169-001	Paratype WAM P.33137-001	Paratype WAM P.33137-001
Standard length	19.5	16	23	16	19
Head length in SL	37.4	36.9	39.6	40.0	40.0
Head depth in HL	54.8	50.8	48.4	48.4	53.9
Head width in HL	61.6	54.2	64.8	57.8	59.2
Body depth at anus in SL	16.4	-	15.2	16.3	18.4
Caud. ped. length in SL	20.5	-	-	21.3	19.5
Caud. ped. depth in SL	9.2	-	-	10.6	10.0
Snout to POP in HL	76.7	74.6	74.7	68.8	75.0
Jaw length in HL	46.6	47.5	44.0	43.8	47.4
Opercle length in HL	31.5	25.4	28.6	28.1	28.9
Pectoral fin in SL	20.0	-	23.0	-	18.4
Pelvic fin in SL	15.4	-	15.2	-	18.9
Caudal fin in SL	28.7	-	18.7	-	28.9

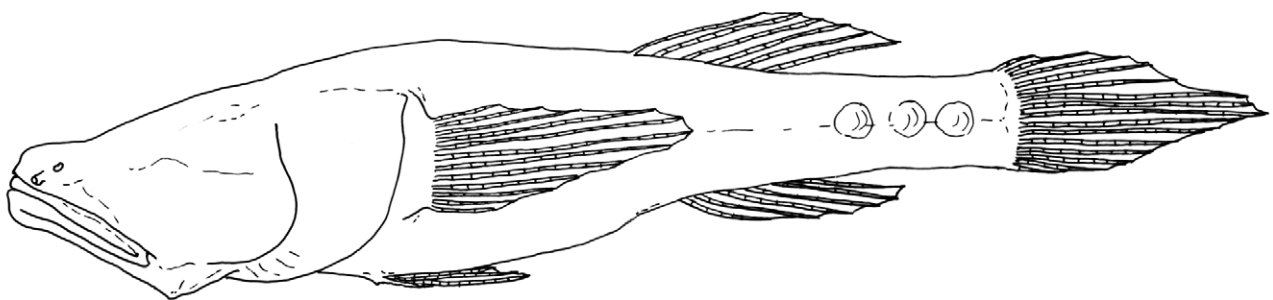


FIGURE 4. Holotype of *Milyeringa justitia* n. sp., WAM P.33166-001, 19.5 mm SL male, Barrow Island, WA. Fins partly reconstructed; five scale pockets not illustrated (three scales remain).

Body mostly naked, cycloid scales in single row from caudal fin base to just below dorsal fin origin; scales embedded in skin, difficult to discern.

First dorsal fin absent. Second dorsal fin with all elements segmented and all rays unbranched; fin rays falling well short of caudal fin base. Anal fin with all rays segmented and unbranched. Pectoral fin somewhat pointed, central rays longest, 18.4–23.0% of SL; all rays unbranched. Pelvic fin length 15.2–18.9% of SL; pelvic spine very short, segmented rays all unbranched; fins very slender, pointed, spine quite short, all segmented rays unbranched,

fins extending about half the distance to anus. Caudal fin oval, pointed posteriorly, central rays may be greatly elongate (often damaged); caudal fin length 18.7–28.9% of SL.

Live coloration. No information available.

Coloration in alcohol. Whitish, with transparent fin membranes (Fig. 6).

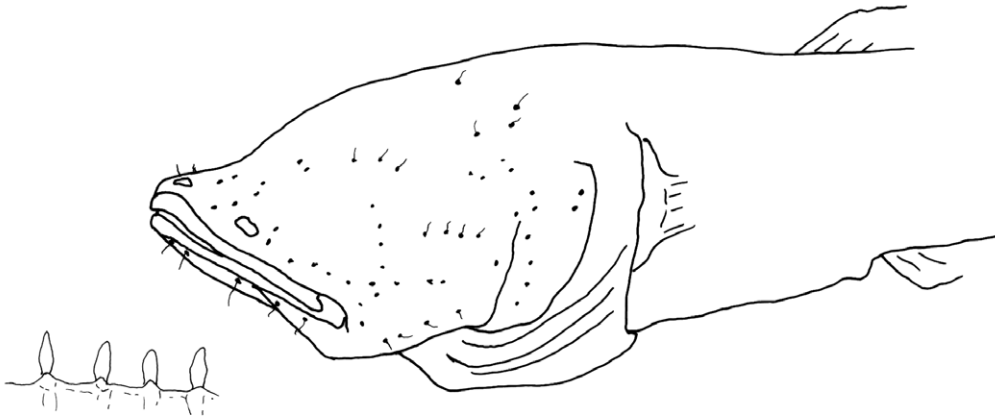


FIGURE 5. Head of *Milyeringa justitia* n. sp., showing sensory papillae pattern and indicating flaps when intact; WAM P.33165-001, head 3 mm long, twisted to left, posterior half of fish missing (taken for genetic sample). Inset shows lateral view of papilla row and flaps.

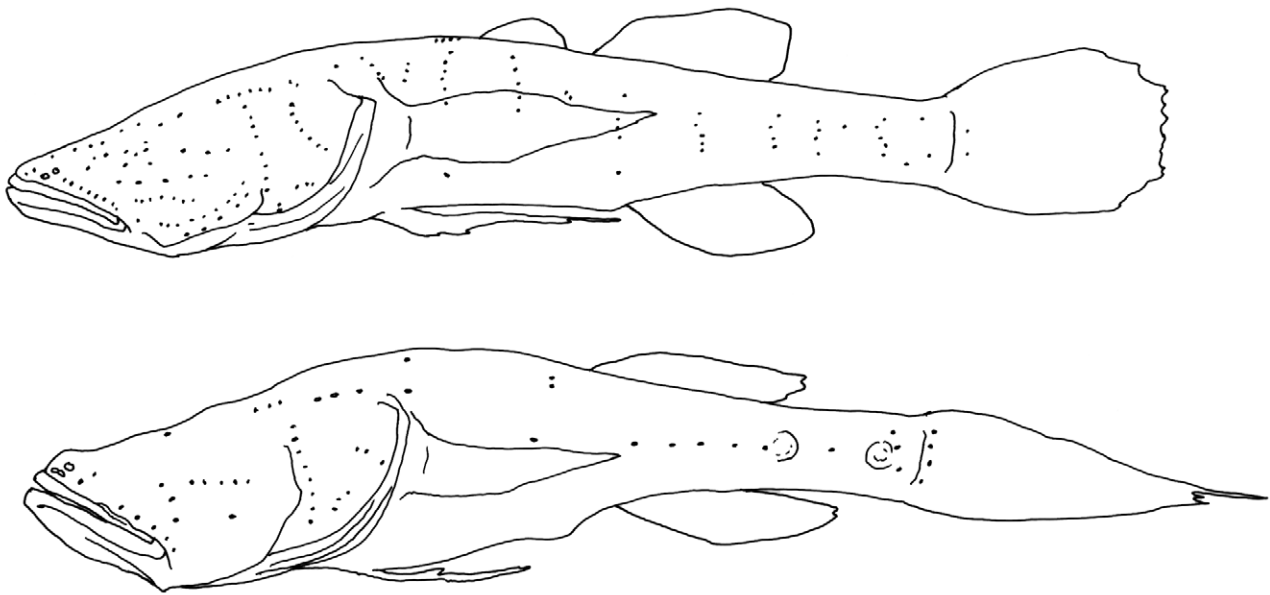


FIGURE 6. Sketch of *Milyeringa veritas* (above) and *Milyeringa justitia* n. sp. (below) showing sensory papillae arrangements on body; *M. veritas* 24 mm SL female, WAM P.33157-001; *M. justitia* n. sp. 19 mm SL male, WAM P.33167-001.

Distribution. This species is known only from Barrow Island, off the north-west coast of Western Australia. No *Milyeringa* has yet been reported from groundwater of the Robe or Fortescue River systems on the mainland east of Barrow Island, despite the presence there of some elements of the typical Cape Range and Barrow Island anchialine fauna, including *Stygiocaris* (Decapoda), *Halosbaena* (Thermosbaenacea), *Haptolana* (Isopoda) and *Ophisternon* (Synbranchiformes) (Humphreys 2008; Page *et al.* 2008).

Comparisons. *Milyeringa justitia* can be separated from *M. veritas* by its lack of a first dorsal fin (*M. veritas* possesses a small first dorsal fin of III–IV spines), in having all dorsal and anal fin elements segmented (*M. veritas* usually has an anal fin spine present), its greatly reduced scalation (with a few cycloid scales along the posterior half of the body compared to the almost complete scalation in *M. veritas*), having fewer sensory papillae on the

head and body (vertical rows on body present in *M. veritas*; Fig. 7), a lower number of segmented caudal fin rays (13–15 in *M. justitia* and 16–17 in *M. veritas*) and a slightly deeper head (mean HD 51.3% of SL in *M. justitia* vs 41.8% in *M. veritas*). *Milyeringa veritas* also has a flatter, wider head in specimens over 30 mm SL, but as the largest *M. justitia* is only 23 mm SL, this feature cannot be compared.



FIGURE 7. Ethanol-preserved paratype of *Milyeringa justitia* n. sp., WAM P.33135-001, from Anode well Q4 on Barrow Island, Western Australia. Photograph by W.F. Humphreys.

Ecology. This species (as *M. veritas*) is protected by state and Commonwealth fauna protection legislation. However, the highly restricted range, with a possible maximum area of occupancy of 78 km² (see Humphreys (2002)), of the new species and potential threats to its habitat warrant specific assessment of extinction risk. That is, it is likely to belong to a higher category of threat than that currently considered for *M. veritas*. The high conservation value of the subterranean fauna of Barrow Island was not recognised until the early 1990s (Humphreys 2001b).

The sparse knowledge of the habitat of *Milyeringa* on Barrow Island was summarised by Humphreys (2001b). *Milyeringa* is known from a former water bore (the type locality) and two anode protection bores in the middle of an oilfield that has been in production since about 1967 and where the water table is up to 54 m below the surface, being 3.8 to 5.8 km from the closest coast (Fig. 8). A tidal range of about three metres, comparable to the ocean tide, in boreholes more than 1 km inland suggests well developed karst below the surface of Barrow Island, an observation that is supported by large voids recorded in drilling logs from oil and anode wells (Humphreys 2001b). The groundwater is an anchialine system showing marked hydrogeochemical stratification (Humphreys 2001b: see Fig. 3 for profile data from the type locality L8 and others), although not as well characterised as that on the Cape Range peninsula (Seymour *et al.* 2007).

Milyeringa justitia is sympatric with a range of stygiobiont crustaceans including Thermosbaenacea, Atyidae (Decapoda), Hadziidae (Amphipoda), Cirolanidae (Isopoda), many copepod taxa including Ridgewayiidae (Calanoida) and it is also sympatric with a groundwater synbranchid eel, apparently an *Ophisternon*, but known only from a photograph, having been delivered to the surface by the detonation of an explosive charge in a seismic exploration well and the specimen was not retained (D. Moro; pers. comm. to WH, 8/9/2009).

Etymology. Whitley chose the name *veritas* for his species because “Truth lies at the bottom of a well” (Whitley 1964). As truth and justice are supposed to go together, we name this species *justitia*, from the Latin for justice, in the hope that justice helps the species to survive on Barrow Island, which has been an oilfield since 1967 and is most recently the site of the Gorgon Gas Hub development.

As *M. veritas* is known as the Cave Gudgeon, we suggest Barrow Cave Gudgeon as a common name for this species.

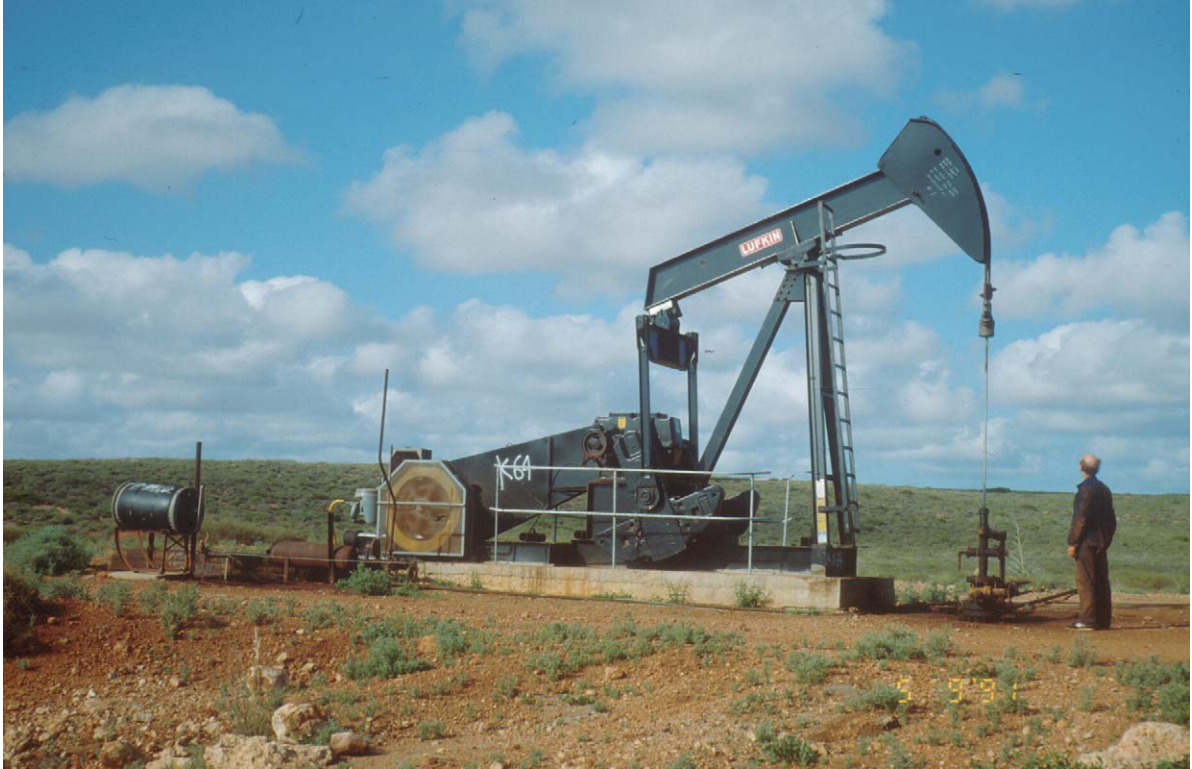


FIGURE 8. Barrow Island beam pump (nodding donkey) about 1 km from well L8 that lies above the habitat of *Milyeringa justitia n. sp.*, in groundwater about 54 m down. Photograph by W.F. Humphreys.

Milyeringa veritas Whitley, 1945

Cave gudgeon

Milyeringa veritas Whitley, 1945: 36–37 (Milyering, Yardie, 20 miles south-west of Vlamingh Head, North-west Cape, Western Australia).

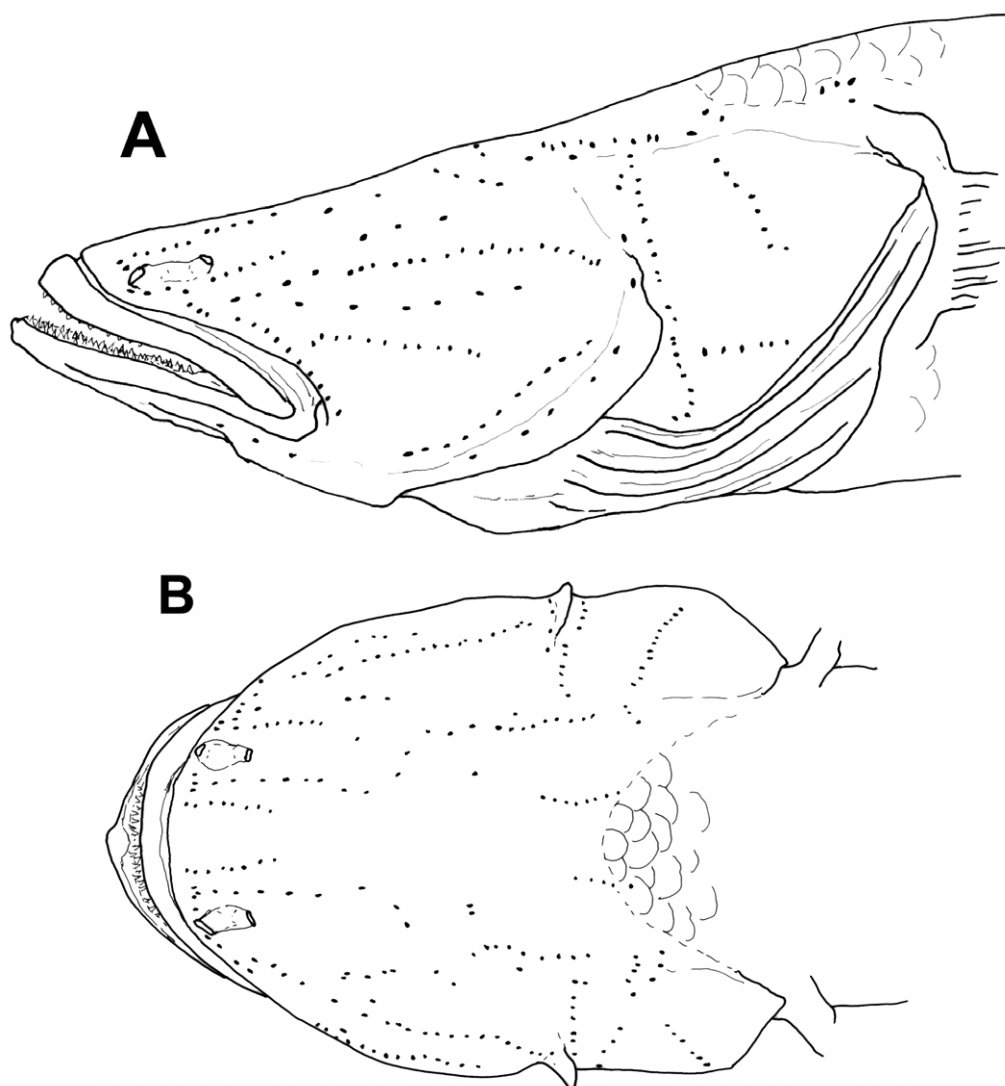
Milyeringa brooksi Chakrabarty, 2010: 22–25 (Pilgonaman Well, North West Cape).

Diagnosis. A *Milyeringa* with small first dorsal fin of II–IV spines, usually III; 6–8 segmented second dorsal fin rays; I,6–8 anal fin rays; pectoral fin rays 11–14; pelvic fin rays I,4; 16–17 segmented caudal fin rays; caudal and pectoral fin rays all unbranched; body fully scaled with scales usually extending forward onto predorsal, 22–29 lateral scales, head short with reduced numbers of rows of sensory papillae on the head and body, posteroventral margin of preopercle forming expanded bony flange; vertebrae 10–12+14–15 (24–26 in total), two pre-anal pterygiophores, one epural. Found in caves and wells of North-West Cape, Western Australia.

Material examined. WESTERN AUSTRALIA: WAM P.2913, holotype of *Milyeringa veritas*, 39 mm SL female, Yardie Station; WAM P.28330-001, holotype of *Milyeringa brooksi*, 36.5 mm SL female, Pilgonaman Well, North-west Cape, M. Newton, 8 July 1984; WAM P.29242-001, paratypes of *Milyeringa brooksi*, 2(36–38.5), Exmouth, B. Vine and party, 19 May 1985; WAM P.28262-001, 8(12.5–40.5), Milyering Well, North-west Cape; WAM P.33161-001, 2(34–36), Kubura Well, Cape Range, W.F. Humphreys, 1 July 1993; WAM P.33157-001, 2(22–24), Five Mile Well, Cape Range, B. Vine, 15 July 1989; WAM P.33168-001, 1(22.5), Exmouth bore field Water Corporation bore 18, Cape Range, Kinhill, 1 April 2001; WAM P.33163-001, 1(13.5), Ampolex site D, Cape Range, R.D. Brooks, 12 November 1995; WAM P.33160-001, 3(31–37), Kubura Well, Cape Range, W.F. Humphreys, 12 August 1993; WAM P.33158-001, 1(32.5), Cape Range, W.F. Humphreys, 15 July 1993; WAM P.33159-001, 2(25–30.5), Kubura Well, Cape Range, W.F. Humphreys, 26 May 1993; WAM P.33164-001, 1(13), Cape Range, A. Poole and S. Eberhard, 22 September 1997; AMS I.25504-001, 1(31), cleared and stained, well 3 km S of Mangrove Bay, Cape Range National Park, D. Hoese and D. Renniss, 13 September 1985.

TABLE 3. Measurements of specimens of *Milyeringa veritas*, expressed as percentage of standard length (SL) or head length (HL). Missing data is usually due to specimen being damaged or distorted. (POP = preopercular posterior margin). N = 24.

	Holotype <i>M. veritas</i>	Holotype <i>M. brooksi</i>	Mean	Maximum	Minimum
Standard length	39.0	36.5	29	40.5	12.5
Head length in SL	40.3	40.0	40.0	42.3	36.9
Head depth in HL	42.7	46.6	42.2	53.5	35.4
Head width in HL	68.2	67.8	61.0	73.7	49.5
Body depth at anus in SL	17.7	17.5	16.9	20.0	12.3
Caud. ped. length in SL	22.8	21.1	24.3	27.7	20.0
Caud. ped. depth in SL	10.0	10.4	10.2	11.8	7.7
Snout to POP in HL	-	71.9	69.1	72.9	65.5
Jaw length in HL	40.1	43.8	37.9	43.8	27.1
Opercle length in HL	-	33.6	34.6	39.8	27.8
Pectoral fin in SL	-	18.6	19.3	28.9	12.3
Pelvic fin in SL	-	19.7	17.0	26.9	7.7
Caudal fin in SL	-	-	28.2	39.2	18.8

**FIGURE 9.** Lateral (A) and dorsal (B) views of head of *Milyeringa veritas*, WAM P.28262-001, 37 mm SL female, showing sensory papillae pattern. Scales indicated.

X-rayed specimens: F03762, 1, North West Cape, West Australia, 21° 47' S 114° 10' E; F05491, 3, township rockhole, Exmouth, West Australia, 21° 56' S 114° 7' E; F05492, 3, rockhole 6 km north of Yardie Creek, West Australia, 22° 20' S 113° 51' E.

Description. Based on 23 specimens, 12.5–40.5 mm SL. Counts and morphometrics of the holotype of *Milyeringa veritas* indicated by asterisk.

First dorsal spines II–IV*, modally III; second dorsal rays 6–8*, modally 8; anal rays I,6–8, modally I,7* pectoral rays 11–14, modally 13; pelvic rays I,4; segmented caudal rays 16–17*, in 8/8 (in 4) or 9/8* pattern (16), all fin rays unbranched; lateral scale count 22–29*, modally 26; transverse scales backward 9–12, modally 10 (11 in holotype); predorsal scale count 0–16*, modally 12; vertebrae 10+14 (1), 11+14 (1), 11+15 (4), 12+14 (1); dorsal pterygiophore pattern 4–221 (in 1); 1 epural; 2 anal pterygiophores anterior to first haemal spine; last haemal spine broadened or forked (with variable amount of sheet of bone between forks).

Body moderate, somewhat rounded anteriorly, compressed posteriorly; body depth at anal fin origin 12.3–20.0% of SL. Caudal peduncle length 20.0–27.7% of SL. Caudal peduncle depth 7.7–11.8% of SL. Head rounded to depressed, larger specimens (over 30 mm SL) with head much wider than deep at preopercular margin, head length 36.9–42.3% of SL; head depth at posterior preopercular margin 35.4–53.5% of HL; width at posterior preopercular margin 49.5–73.7% of HL. Mouth large, terminal and oblique, chin tip anteriormost, jaws forming an angle of about 35° with body axis. Upper jaw length 27.1–43.8% of HL; inner margin of lips smooth; lower lip fused to chin anteriorly, side of lip free; chin flat with no mental frenum. Anterior naris in short tube at edge of or just above upper lip; posterior naris oval to flattened oval, with low rim (or very short tube), occasionally anterior rim slightly produced as low curved flap; nares joined by thin “tube” over nasal rosette. Eyes absent. Snout area broad, flattened, tilted upward slightly, rounded to nearly square anteriorly in dorsal view; snout slightly pointed in lateral view mostly in small specimens, length of snout tip to upper edge of the preopercular margin 65.5–72.9% of HL. Opercle length (upper edge of preopercle to upper margin of opercle) 27.8–39.8% of HL. Preopercular margin bony and broadened into flange posteroventrally, extending from about halfway down posterior margin nearly to rictus; flange present even in small specimens (e.g. 15 mm SL), gently curved outward in some specimens (may not be size-related), flange may be angular or with indentation near corner. Gill opening wide and somewhat variable in anterior extent, reaching forward from just before preopercular rear margin (as in holotype) to halfway between preopercular rear margin and rictus. Tongue large, tip blunt. Teeth in both jaws small, evenly sized, conical and pointed; in two to three rows; teeth of inner rows larger, pointing inward in larger specimens and more upright in smaller. Headpores absent. Sensory papillae conspicuous fleshy bumps, in longitudinal pattern; papillae with thin narrow flap each; thin flaps usually present only on well-preserved specimens (Fig. 9). Sensory papillae on body on body in variable numbers of irregular vertical (transverse) rows, often interspersed with single papillae and few papillae along hypural crease; papillae on body sometimes with broad to thin narrow flaps (uncertain if absence actual or due to damage); three longitudinal rows of papillae on caudal fin (Fig. 10).

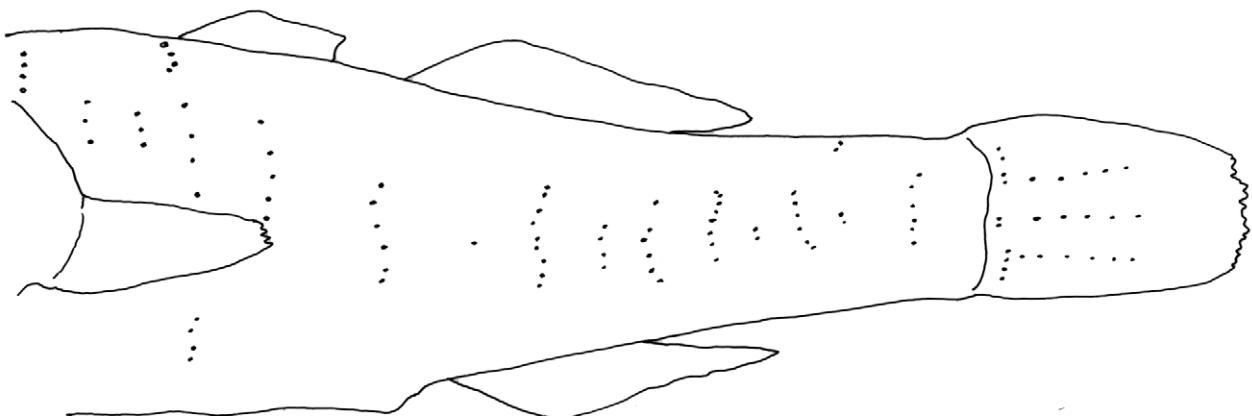


FIGURE 10. Composite sketch of *Milyeringa veritas* showing papillae rows on body and caudal fin; taken from WAM P.28262-001, 40 mm SL male, and WAM P.133158-001, 32.5 mm SL probable female.

Body covered with small cycloid scales, reaching forward onto head above level of opercle or above rear preopercular margin. Side of head naked. Prepelvic region naked (usually) or with patch of cycloid scales just before pelvic fin bases. Pectoral fin base naked. Belly covered with cycloid scales.

First dorsal fin greatly reduced, roughly triangular, with gap of about three or four scales before the second dorsal fin origin. Posteriormost second dorsal and anal fins short-based, rays taller than first dorsal fin; posterior rays slightly longer than anterior rays but not greatly so, posterior rays falling well short of caudal fin base. Second dorsal fin with all elements segmented. Anal fin usually with spine and segmented rays (three specimens with all elements segmented). Pectoral fin small, slender, pointed, central rays longest, 12.3–28.9% of SL; all rays unbranched. Pelvic fin length 7.7–26.9% of SL; fins small, very slender and pointed, fins extending less than half the distance to anus. Caudal fin oval, with central rays elongate, often greatly so (elongate rays often damaged or broken in available specimens); caudal fin length 18.8–39.2% of SL.

Live coloration. Photos of living fish are shown in Allen (1982), Merrick and Schmida (1984), Young (1986) and Allen *et al.* (2002) (Fig. 11). Fish white to translucent white posteriorly, with transparent fins, and red gills and pinkish colour from abdominal organs showing through opercle and body wall.



FIGURE 11. Captive *Milyeringa veritas*. Photograph by Douglas Elford, Western Australian Museum.

Coloration in alcohol. Whitish, with transparent fin membranes. Specimens often with very fine scattered blackish to dark brown melanophores over dorsal surface of cranium and frontals (i.e. would-be interorbital space).

Results of genotyping. The branch of the recovered COI gene tree representing mainland (Cape Range peninsula) *Milyeringa* closely resembles Chakrabarty's (2010) tree in topology and genetic distances within and between two major clades (Fig. 3) though greater substructure is evident. Most significantly, however, we found both major mitochondrial lineages co-occurring at C-25, Kudamurra Well (samples in blue in the NJ tree, Fig. 3). This population shows no concordant allozyme differentiation between individuals of 'veritas' haplotype and those of 'brooksi' haplotype (Adams and Humphreys 1993), a finding consistent with the presence of free gene flow and a single species, *Milyeringa veritas*.

Distribution. Known from Cape Range peninsula, Western Australia.

Ecology. *Milyeringa veritas* is listed as vulnerable under the *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth) and as endangered under Schedule One of the *Wildlife Conservation Act 1950* (Western Australia) owing to its small geographic distribution, low populations and vulnerability of its habitat. Two locations in which the species occurs (Bundera Sinkhole and Camerons Cave) are also listed as threatened communities (by the Western Australian Government's Department of Environment and Conservation). Its distribution lies within the Ningaloo Coast World Heritage Area. The conservation status is discussed by Romero and Vanselow (2000).

The sparse knowledge of the biology of *Milyeringa* was summarised by Humphreys (2001a). *Milyeringa veritas* inhabits the coastal groundwaters of the carbonate karst of the Cape Range peninsula that projects onto the North West Shelf of Australia. It occurs on the coastal plain and foothills of the peninsula between 0.4 km and 4.0 km of the coast. Closer to the coast is an anchialine system, characteristically with marked hydrogeochemical stratification with depth (Seymour *et al.* 2007), but grading to freshwater inland. The fish occupy a wide range of salinity, from freshwater inland to fully marine in the depths of the anchialine system (Humphreys 2001a). Otolith microchemistry indicates that individuals may inhabit waters of widely different salinity through their lives (Humphreys *et al.* 2006). They seem to be opportunistic feeders ingesting prey of epigeal origin as well as obligate groundwater species (Humphreys & Feinberg 1995).

Anchialine habitats of the type inhabited by *Milyeringa* are noteworthy for their very diverse stygiobiont crustacean fauna, comprising a characteristic assemblage of higher taxa, the structure of which is highly predictable, frequently extending to the generic composition (Wagner 1994), however far apart in the world they occur (Jaume *et al.* 2001); most of their members represent biogeographic and/or phylogenetic relicts (Iliffe 1992). As such, the anchialine fauna of the Cape Range peninsula has an assemblage comparable to anchialine systems of the North Atlantic comprising, *inter alia*, the higher taxa Remipedia, Thermosbaenacea, Atyidae (Decapoda), Hadziidae (Amphipoda), Cirolanidae (Isopoda), Thaumatocypridae (Ostracoda), and the copepod taxa Calanoida (Epactericiscidae, Ridgewayiidae and Pseudocyclopiidae), Misophrioida (Speleophriidae) and Cyclopoida (Halicyclopiinae). *Milyeringa veritas* is sympatric with the groundwater synbranchid eel *Ophisternon candidum* (Mees, 1962).

Remarks. *Milyeringa brooksi* was described by Chakrabarty (2010), who separated his species from *M. veritas* mainly by its having 10–12 vertical lines of sensory papillae along the body and by “molecular synapomorphies” (which refer to changes in nucleotide position in three genes: ND2, CytB and COI). Chakrabarty also considered that *M. brooksi* was a smaller fish than *M. veritas* (the largest specimen given as 38.33 mm SL versus 52.6 in *M. veritas*), had a tubular posterior nostril with a skin flap (versus a simple round nostril in *M. veritas*) and had conspicuous papillae on the dorsal surface of the head (versus a “variable condition” in *M. veritas*). The nostril size and shape in the holotype of *M. brooksi* and the two WAM paratypes are basically the same as in *M. veritas*. We cannot distinguish his specimens as a separate species from *M. veritas*.

We observed features in *Milyeringa* specimens that were missed by Chakrabarty (2010). He indicated that he had 16 specimens of *M. veritas* (sizes given as 30.7–52.6 mm SL) to compare with his seven specimens of *M. brooksi*. He apparently did not observe the vertical rows of sensory papillae along the side of the body in *M. veritas* which are present in specimens of all sizes and locations. He also describes the head of *M. brooksi* as being naked, although scales can be observed on the holotype’s predorsal region, extending forward to above the preopercular margin.

Chakrabarty’s 2010 DNA sequence data for *Milyeringa* were derived from eight specimens from seven populations. The maximum genetic distance found between the two major mitochondrial lineages that represent *M. veritas* and *M. brooksi* was 3.2%. The small sample size and low genetic distance (in comparison with genetic distances found among other eleotrid species; e.g. Stevens & Hicks 2009, Thacker 2009) are themselves problematic but, as highlighted in recent discussions of the use of barcoding (e.g. Lohse 2009; Petit & Excoffier 2009), the use of markers from a single uniparentally inherited genome for species delimitation is risky and the inference of a species tree from what is effectively a single gene tree, inadvisable. Multilocus assignment methods, using unlinked markers, have considerably more power in this respect and can also inform on population admixtures.

Humphreys and Adams (1991) and Adams and Humphreys (1993) subjected seven populations of *Milyeringa veritas* ($n = 24$ & 29 , respectively) to comprehensive allozyme analyses at 43 loci and demonstrated that, although allele frequencies and distributions indicated significant population sub-structuring on the peninsula, overall levels of differentiation and the pattern of allele distribution were consistent with the presence of a single biological species on Cape Range peninsula. The mitochondrial data presented here affirm the view established in the 1990s and provide additional evidence for the distinctiveness of the species from Barrow Island.

Conclusions. Cape Range peninsula and Barrow Island are home to a distinctive and unique stygofauna including cave-dwelling fishes. The description of a new and highly divergent *Milyeringa* from within the range of an already restricted taxon, adds significantly to the regional fauna with matching conservation implications, in the face of increasing and broad-scale mineral exploration. We recognise that further systematic work may be required to understand the evolutionary heritage of *Milyeringa* species.

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