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Description of a freshwater eeltail catfish (Teleostei: Plotosidae) from sandstone habitat in the eastern Kimberley, Western Australia

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Abstract

The Kimberley region of north-western Australia is an exceptional region for endemic freshwater fishes. During a survey program in partnership with local land and sea rangers in the eastern Kimberley in 2014, an unusual morphotype of an eeltail catfish (Plotosidae) was recorded, featuring a moderately extended caudodorsal fin, small first dorsal fin, hard sharp fin spines and depressed head, collectively representing a unique combination of characters. Nuclear and mitochondrial genetic assessments confirmed the presence of a distinct candidate species. Combined lines of evidence are used to describe *Neosilurus manjandi* sp. nov. The recorded habitat consists of sandstone upland streams and gorges in the Pentecost and Drysdale river catchments. Preliminary genetic data for Kimberley freshwater plotosids reveal the presence of several other cryptic lineages that warrant further genetic and taxonomic assessments. Maintaining refuge pools is a likely key conservation consideration for the new species in the face of any future catchment modification and climate change.

Key words: morphology, taxonomy, genetics, freshwater biodiversity, allozymes, mtDNA, *Neosilurus*

Introduction

The remote Kimberley bioregion of north-western Australia is dominated by rugged and spectacular sandstone escarpment, containing a naturally fragmented and ancient landscape with distinct and isolated river systems. It has a rich Aboriginal culture and diverse and endemic biota, with the freshwater fish fauna containing at least 65 species, nearly half of these being endemic to the region (Unmack, 2001; Allen *et al.*, 2002; Morgan *et al.*, 2011; Shelley *et al.*, 2018a). Eeltail catfishes (family Plotosidae) are a significant component of the Kimberley freshwater fish fauna, with five species recorded in the region (i.e. 7.7% of the fauna). These eeltail catfishes, as currently defined, have ranges extending beyond the Kimberley, with variable distribution patterns and several species often occurring sympatrically: Hyrtl's Catfish *Neosilurus hyrtlii* Steindachner 1867 is the most widespread, being ubiquitous across the region; the Softspined Catfish *Neosilurus pseudospinosus* Allen & Feinberg 1998 is restricted to more perennial upland stream and riffle habitats; the Black Catfish *Neosilurus ater* (Perugia 1894) prefers larger waterholes and is spread patchily in the landscape; Rendahl's Catfish *Porochilus rendahli* (Whitley 1928) is mainly a wetland species associated with aquatic vegetation in the lower portion of river systems; and the Toothless Catfish *Anodontiglanis dahli* Rendahl 1922 has a very fragmented and restricted distribution, being recorded in low abundance from parts of the Fitzroy River system and the Keep/Victoria rivers (Allen and Feinberg, 1998; Allen *et al.*, 2002; Morgan *et al.*, 2011; Pusey *et al.*, 2017; Shelley *et al.*, 2018a).

Field sampling in the Kimberley bioregion continues to yield undescribed species and major range extensions (Morgan *et al.*, 2011; Shelley *et al.*, 2017; Shelley *et al.*, 2020; Shelley *et al.*, 2023). During a Bush Blitz survey (a national nature discovery program) to the eastern Kimberley region within the Pentecost River watershed (Moore and Hammer, 2015; Commonwealth of Australia, 2016; Fig. 1), a previously undocumented form of plotosid was encountered. It is most similar to *N. ater*, sharing hard spines and a moderately extended caudodorsal fin, but differs in general shape (small first dorsal fin, depressed head, shallow body depth) and colouration (dark body with contrasting yellowish caudodorsal fin). Herein, we describe the species using combined lines of evidence assessing congruence between co-dominant nuclear markers (allozymes), mitochondrial DNA sequence divergence data (*cytb* gene), morphology (suite of 33 meristic and morphometric characters), and ecology (Page *et al.*, 2005; Adams *et al.*, 2014; Hammer *et al.*, 2019). The most recent taxonomic review of Australian freshwater plotosids was by Allen and Feinberg (1998) who described a new genus (*Neosiluroides*) and three species of *Neosilurus*, and provided general comparative information for all freshwater genera in Australia-New Guinea (a broader collation of morphological data is also provided in Allen *et al.*, 2002). Two species of the freshwater genus *Tandanus* have been described since (Welsh *et al.*, 2014; Welsh *et al.*, 2017), along with revisions of the marine genera *Euristhmus* (Murdy and Ferraris, 2006) and *Paraplotosus* (Allen, 1988).

Methods

Study material

Tissue vouchered material of the new species and additional plotosids were collected during the East Kimberley Bush Blitz survey covering the Durack River and Karunjie stations in the Pentecost River Catchment of the East Kimberley (Fig. 1; Moore and Hammer, 2015). Voucher samples were fixed in 10% formalin, rinsed and stored in ethanol, and lodged at Western Australian Museum, Perth (WAM) and Museum and Art Gallery of the Northern Territory, Darwin (NTM). Prior to fixation, a small piece of muscle was removed from the right side of the fish, and snap frozen in liquid nitrogen. Tissues were transported to the Australian Biological Tissue Collection (ABTC) at the South Australian Museum (SAMA) and stored at -70°C . Comparative frozen tissues for other Kimberley *Neosilurus* were also available in the ABTC from opportunistic sampling by the authors across northern Australia, forming the basis for a comparative dataset of related congeners using allozymes (which require frozen tissue). A complementary mitochondrial dataset was established following the allozyme species framework and expanded upon

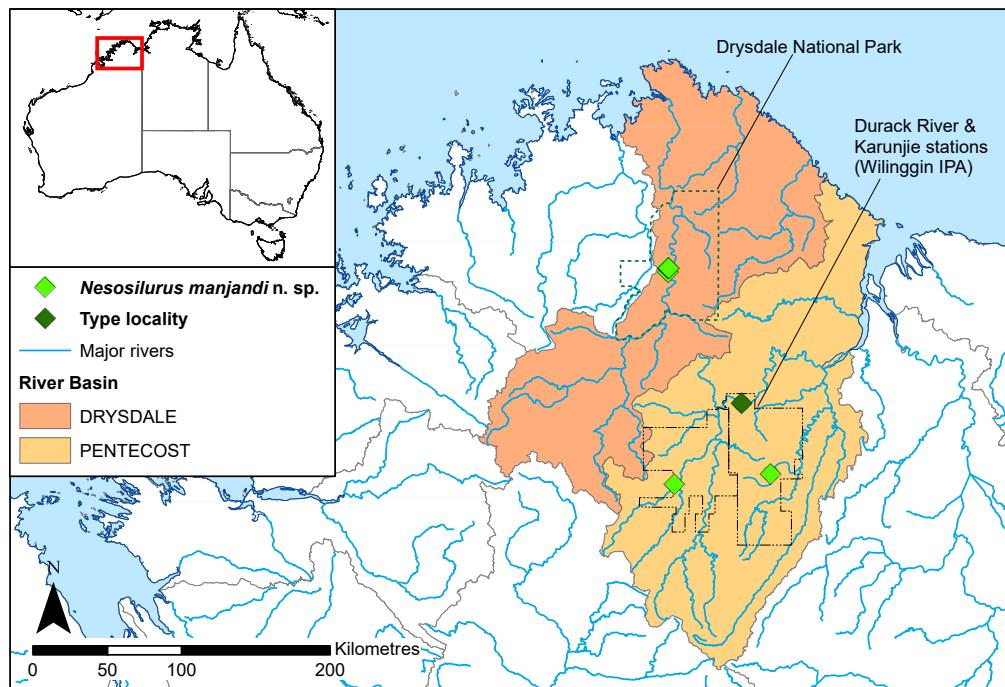


FIGURE 1. Distribution of the Sandstone Catfish *Neosilurus manjandi* sp. nov. in the Kimberley bioregion, Australia.

through broader taxon sampling to represent the northern Kimberley freshwater plotosid fauna. This was intended as an overview study rather than as a systematic revision of each species. This included ethanol-fixed tissues, or whole ethanol-fixed animals sourced from colleagues, including sampling by Murdoch University (DM site codes, see Morgan *et al.*, 2011), Melbourne University (NMV site codes, see Shelley *et al.*, 2018b) and Australian Museum, Sydney (AMS site codes). Museum collections in Australia were physically searched to identify any other study vouchers and to verify identifications of Kimberley region material, which resulted in the identification of further formalin fixed material (no tissues) of the new species from the Drysdale River catchment present in both WAM and Museum Victoria, Melbourne (NMV).

Molecular genetics

The compiled nuclear genetic dataset consists of allozyme profiles generated from muscle homogenates. All methodological details, including enzyme commission numbers, electrophoretic conditions, histochemical stain recipes, and locus/allozyme nomenclature have been described previously (Richardson *et al.*, 1986; Hammer *et al.*, 2007). The following enzymes or non-enzymatic enzymes displayed allozymically-interpretable patterns: ACON, ACP, ACYC, ADA, ADH, AK, ALD, CA, CK, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GP, GPI, IDH, LDH, MDH, ME, MPI, NDPK, NP, PGAM, 6PGD, PGK, PGM, PK, PEPA, PEPB, PEPD, TPI, and UGPP. Initially, Principal Co-ordinate Analysis (PCoA) was used to independently identify the genetic affinities of individual fish, without *a priori* reference to any geographic and taxonomic expectations. Thereafter, a pairwise matrix of the number of fixed differences and unbiased Nei Distances was calculated for the primary genetic groupings identified via PCoA. The details of both analytical approaches have been published elsewhere (Adams *et al.*, 2014; Hammer *et al.*, 2014), with our general rationale for assessing species boundaries discussed in Unmack *et al.* (2022).

Matrilineal genetic sequence data (mtDNA) were obtained from the whole cytochrome *b* (cytb) gene following the extraction, sequencing and editing protocols of Unmack *et al.* (2009). Sequences coding for amino acids were aligned by eye and checked via amino acid coding in MEGA 7.0.18 (Kumar *et al.*, 2016) to test for unexpected frame shift errors or stop codons. Phylogenetic analyses of the final sequence dataset employed maximum likelihood (ML), as implemented via IQ-TREE 1.6.12 (Nguyen *et al.*, 2015) run on the W-IQ-TREE server (Trifinopoulos *et al.*, 2016). These analyses employed IQ-TREE's model selection procedure (-m TEST+ASC; Kalyaanamoorthy *et al.*, 2017), resulting in the selection of the TPM2+F+I+G4 model followed by 10,000 replicates of ultrafast bootstrapping (-aLRT 10000; Hoang *et al.*, 2018). Average intra and inter-specific p-distances were calculated using MEGA. All sequences generated in this study were deposited in GenBank with accession numbers PQ480884–PQ480910.

Morphology

A statistical comparison of meristic values was undertaken using data obtainable from x-rays in order to verify gross diagnostic characters between Kimberley *Neosilurus* species, which have been previously reviewed (Allen and Feinberg, 1998; Allen *et al.*, 2002), and the new species (i.e. individual count data for vertebrae and non-paired fin elements in support of broader published ranges). The coarse meristic data were explored using XLSTAT 2016.1.01 (Addinsoft TM) employing Principal Component Analysis (PCA), using Pearson Correlation matrix, Varimax rotation, and with correlations between factor scores and characters displayed as vectors. A specific detailed assessment of specimens representing the new species was then undertaken to obtain descriptive data. Measurements were taken using digital callipers and aided with a dissecting microscope where necessary. Morphological methods follow Allen and Feinberg (1998), with a full review and standardisation/documentation of characters undertaken to provide a standard methodology for future taxonomic endeavours due to inconsistencies or lack of details by previous authors (see Table 1). Non-paired fin element and vertebrae counts were taken from x-rays.

The somewhat unique body form of plotosid catfishes has created variable use of fin terminology. Here, we recognise all the continuous fin elements of the eel-like tail to represent a caudodorsal fin, representing the confluent: (a) second dorsal fin which is technically the elongated dorsal procurent caudal fin rays rather than strictly a separate fin, but we favour the practical label of second dorsal fin as advocated by Murdy and Ferraris (2006) and used in regional freshwater field guides (Allen *et al.* 2002; Shelley *et al.* 2018a); (b) fused caudal fin, with rays defined as

only those rays fully articulating basally with the hypural plate as best that could be determined depending on x-ray and preservation quality; and (c) anal fin, including all procurent rays and rays with pterygiophores anterior to the hypural plate. The small anterior elements and origin of the second dorsal fin require close examination, while the ultimate ray of the first dorsal fin is paired at the base and counted as one element (Fig. 2). The vertebral count excludes fused elements of the Weberian apparatus and includes the urostyilar vertebra, with precaudal elements distinguished by those having plural ribs falling anterior to the first anal pterygiophore. Paired-fin element counts were mostly taken directly from the specimens, it being necessary to cut and peel back the skin in larger individuals; smaller animals required x-ray verification of rays, with spreading the paired fins and aligning the body at an angle slightly offset from the vertical dorsal view producing the best results. A technique to measure fragile and often curled barbels was developed whereby the barbels were carefully straightened onto a small piece of x-ray film, with

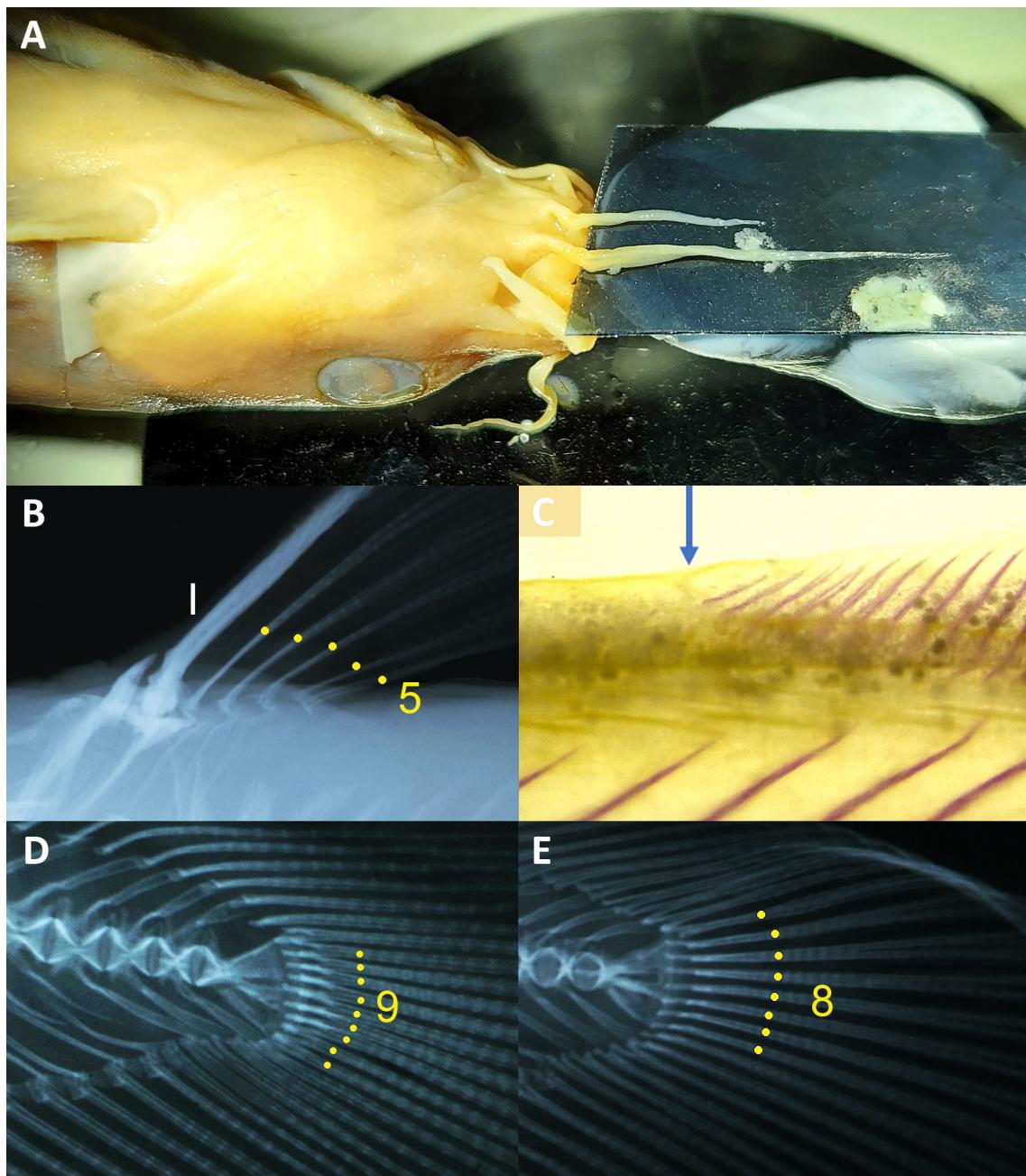


FIGURE 2. Methods used for morphological data collection in plotsid catfishes, demonstrated with *Neosilurus manjandi* sp. nov.: (A) carefully straightening barbels for measurement using an adjustable bench (x-ray film and plasticine); (B) counting first dorsal fin rays including the last ray branched to the base (radiograph); (C) details of the anterior-most dorsal extension (insertion) and small procurent rays of the caudodorsal fin (cleared and stained specimen); and (D + E) counting caudal fin rays articulating fully with the hypural plate (radiographs: WAM P.34043-011 and NTM S.17750-001 respectively).

the height and angle of the film manipulated using a piece of plasticine to facilitate full barbel extension (Fig. 2). Type material is deposited at WAM, NTM and NMV. Select abbreviations: HL, head length; SL, standard length; TL, total length.

TABLE 1. Morphometric characters recorded for plotosid catfishes.

Character	Notes on measurements
<i>After Allen & Feinberg (1998)</i>	
Head length	Snout tip to upper rear corner of operculum
Body depth	Vertically from first dorsal fin origin
Preanal length	Snout tip to anal fin origin
Caudodorsal fin length	First upper procurrent ray to base of middle rays (origin verified with transmitted light or x-rays)
Anal fin length	First anal fin ray to base of middle caudal fin rays
Snout length	Snout tip to anterior edge of eye
Eye diameter	Horizontally across middle of eye
Interorbital width	Least width between bony orbits
First dorsal fin height	Base of fin spine to tip of longest ray
Pectoral fin length	Base of fin spine to tip of longest ray
Pelvic fin length	Base of first (outermost) ray to tip of longest ray
<i>Additional characters</i>	
Head width/height	At point where ventral body surface intersects with gill cover (in lateral view)
Barbel lengths	From base to tip (straightened onto a flat surface)
Predorsal length	Snout tip to first dorsal fin origin
Predorsal-caudal length	Snout tip to caudodorsal fin origin

Results

Molecular data

The final allozyme dataset comprised genotypes for 42 fish (Table 2) at 51 putative loci (Table 3). Visual examination of the raw data for individuals in ordination space (PCoA: Fig. 3a) identified five primary groupings (clusters), corresponding to *N. ater*, *N. hyrtlpii*, two groups of *N. pseudospinosus* (east = Ord River samples, west = Drysdale River samples; note this is only partially representative of a much broader geographic distribution in the Kimberley for this taxon), and the new species, plus an intermediate individual consistent with being an F_1 hybrid between the new species and *N. pseudospinosus* east (supported by examination of raw allele profiles: Table 3). The five primary groupings were strongly supported by fixed differences (FD) at 11–19 loci (Table 4), well above the nominated species-level yardstick (3–5 FD depending on geographic scenario), with the key observation being the new species was readily diagnosable from regional congeners at multiple co-dominant nuclear markers. Considerable spread was noted within the *N. hyrtlpii* cluster, with a follow-up ordination suggestive of three geographic groups (Fig. 3a inset), namely western (lower Fitzroy, Lennard and Isdell catchments), central (upper Fitzroy, Prince Regent and Drysdale catchments) and eastern (Durack, Ord and Victoria catchments), albeit based on low sample sizes and serving as an initial indication of potential species-level structure (5–6 FD: Table 4).

The mtDNA *cytb* dataset comprised 1138 bp of sequence data for 28 individuals, representative of broad geographic representation within all Kimberley plotosids, plus comparative material from further east and a marine outgroup (Table 2). Of these, 707 bp were constant, 73 variable characters were parsimony uninformative, and 358 characters were parsimony informative. *Anodontiglanis dahli* had a premature stop codon on the last complete triplet of *cytb* (usually the stop codon is created by the addition of AA at the end of the mRNA when *cytb* gets translated). The ML tree (-ln score of -5461.387800, Fig. 4) displays the relationships of these individuals. All deeper nodes had moderate to strong support based on bootstrap values >70 . Mean within and between species p-distances are shown in Table 5. All the Kimberley plotosids (i.e. also including *A. dahli* and *P. rendahli*) were represented by distinct

TABLE 2. Sample details for Kimberley photosid catfishes for combined genetic and morphological investigations.

ID	Taxon	Locality	State	River system	Field code	Latitude	Longitude	mtDNA	Morphology	Tissue code	Vouchers examined	
C001	<i>Anodontiglanis dahli</i>	Lake Skeleton	WA	Fitzroy	DM01-18	-17.8754	123.6911	1	DM 18			
C002	<i>Anodontiglanis dahli</i>	Lower Daly River	NT	Daly	MH16-05	-14.1158	131.2864	1	NTM A 05187			
C003	<i>Anodontiglanis dahli</i>	South Alligator River	NT	Alligator	JB88-01	-13.2960	132.3360	1	NTM 474			
C004	<i>Anodontiglanis dahli</i>	Maryfield Creek	NT	Roper	DW21-MC01	-15.4630	133.7560	1	NTM A 08572			
C005	<i>Neosilurus ater</i>	Giekie Gorge	WA	Fitzroy	MA08-47	-18.1173	125.6749	4	ABTC 13337-43			
C006	<i>Neosilurus ater</i>	King Edward River	WA	King Edward	GM63-01	-14.2970	126.6370	2	WAM P.8493-001			
C007	<i>Neosilurus ater</i>	Lawley River	WA	Mitchell	MIT76-17	-14.6885	125.9533	3	WAM P.25677-008			
C008	<i>Neosilurus ater</i>	Berkley River	WA	Berkley	JSM21-BR	-14.5148	127.6220	1	NMV A.311774-005			
C009	<i>Neosilurus ater</i>	Moonshine Gorge	WA	Pentecost	MH13-33	-16.0441	128.0099	1	NTM A 02440	NTM S.17671-016		
C010	<i>Neosilurus ater</i>	Limestone Creek	NT	Victoria	MH15-10	-16.0496	130.3976	2	1	NTM A 03780-81	NTM S.17917-010	
C011	<i>Neosilurus ater</i>	Lower Daly River	NT	Daly	MH12-60	-13.8619	131.0740	2	NTM A 00688-9			
C012	<i>Neosilurus ater</i>	Angurugu Creek	NT	Groote	GE21-06	-13.9835	136.4813	1	NTM A 08426			
C013	<i>Neosilurus ater</i>	Mogunda Creek	NT	Roper	MH12-28	-14.1873	134.3720	2	2	NTM A 00395-6	NTM S.17368-004	
C014	<i>Neosilurus ater</i>	Coleman River	QLD	Coleman	MH15-38	-14.8484	142.5401	2	1	NTM A 04037-8	NTM S.13939-010	
C015	<i>Neosilurus ater</i>	Gap Creek lower	QLD	Daintree	BE16-Gap	-15.8320	145.3320	1	NTM A 05773			
C016	<i>Neosilurus ater</i>	Lagoon Creek	QLD	Murray	PU01-42	-18.0617	145.9084	1	ABTC 77567			
C017	<i>Neosilurus hyrtlii</i> (west)	Bell Creek	WA	Isdell	MA08-41	-17.0168	125.2298	2	ABTC 13314+6			
C018	<i>Neosilurus hyrtlii</i> (west)	Dillie Gorge	WA	Isdell	JSM13-ID	-16.7345	125.3830	2	NMV A.31746-002			
C019	<i>Neosilurus hyrtlii</i> (west)	Brooking Creek	WA	Fitzroy	MA08-48	-18.1788	125.5724	1	ABTC 13355			
C020	<i>Neosilurus hyrtlii</i> (west)	Tunnel Creek	WA	Lennard	JP92-n05	-17.6080	125.1460	2	AMS EBU21561+3			
C021	<i>Neosilurus hyrtlii</i> (central)	Pitta Creek	WA	Prince	JSM13-PRP	-15.8534	125.6497	1	ABTC 2D768			
C022	<i>Neosilurus hyrtlii</i> (central)	Barnett River	WA	Fitzroy	MA08-36	-16.7067	125.9357	2	1	ABTC 13275+7		
C023	<i>Neosilurus hyrtlii</i> (central)	Above Mitchell Falls	WA	Mitchell	JSM12-MF	-14.8195	125.6910	1	NMV A.31722-014			
C024	<i>Neosilurus hyrtlii</i> (central)	Miners Pool	WA	Drysdale	JP92-n02	-15.6817	126.4050	2	1	AMS EBU21334+6		
C025	<i>Neosilurus hyrtlii</i> (central)	Russ Creek	WA	Drysdale	JSM12-DRR	-16.0456	126.7020	1	1	NMV A.31752-018		

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TABLE 2. (Continued)

ID	Taxon	Locality	State	River system	Field code	Latitude	Longitude	Tissue code	Vouchers examined	
Morphology										
mtDNA										
C026	<i>Neosilurus hystrii</i> (central)	mid-Drysdale River	WA	Drysdale	JSML13-DRM	-15.0061	126.9169	1	NMV A.31775-007	
C027	<i>Neosilurus hystrii</i> (east)	Horse Creek	WA	Pentecost	BBK14-003	-16.2526	127.5343	1	2	WAM P.34035-010
C028	<i>Neosilurus hystrii</i> (east)	Bamboo Creek	WA	Pentecost	MA08-30	-15.8723	127.3509	2	ABTC 145766-7	WAM P.34033-004
C029	<i>Neosilurus hystrii</i> (east)	Bindoola Creek	WA	Pentecost	BBK14-001/13	-15.7644	127.7161	2	ABTC 145632+4	WAM P.34044-003
C030	<i>Neosilurus hystrii</i> (east)	Un-named creek	WA	Ord	MA08-21	-17.3867	128.1564	1	ABTC 145632+4	WAM P.34044-003
C031	<i>Neosilurus hystrii</i> (east)	Salmond River	WA	Pentecost	BBK14-012	-16.2786	127.6977	2	ABTC 145632+4	WAM P.34044-003
C032	<i>Neosilurus hystrii</i> (east)	Limestone Creek	NT	Victoria	MH15-12	-16.0461	130.3820	1	NTMA 03814	WAM P.34044-003
C033	<i>Neosilurus mollespiculum</i>	Blue Range	QLD	Burdekin	PU15-45	-19.1701	145.4284	1	NTMA 04235	WAM P.34044-003
C034	<i>Neosilurus pseudospinosus</i>	Calder River	WA	Isdell	JSM14-CR	-16.0549	125.2140	1	1	NMV Z74464
C035	<i>Neosilurus pseudospinosus</i>	Charnley River	WA	Isdell	JSM14-CR	-16.4002	125.1570	1	1	NMV Z74467
C036	<i>Neosilurus pseudospinosus</i>	Dillie Gorge	WA	Isdell	JSM13-ID	-16.7345	125.3830	2	2	NMV A.31746-004+5
C037	<i>Neosilurus pseudospinosus</i>	Manning Gorge	WA	Fitzroy	JSM12-MG	-16.6554	125.9270	1	1	NMV Z74211
C038	<i>Neosilurus pseudospinosus</i>	Carson crossing	WA	King Edward	JSM14-CR	-14.4518	126.6620	2	2	NMV A.31777-003
C039	<i>Neosilurus pseudospinosus</i>	Miners Pool	WA	Drysdale	JP92-m02	-15.6817	126.4050	2	2	AMSEBU21338+40
C040	<i>Neosilurus pseudospinosus</i>	Russ Creek	WA	Drysdale	JSML12-DRR	-16.0456	126.7020	2	2	NMV A.31752-002
C041	<i>Neosilurus pseudospinosus</i>	Drysdale crossing	WA	Drysdale	JSML13-DRC	-14.4472	126.8600	2	2	NMV A.31947-001
C042	<i>Neosilurus pseudospinosus</i>	Horse Creek	WA	Pentecost	MH14-10	-16.2526	127.5343	1	3	WAM P.34035-012
C043	<i>Neosilurus pseudospinosus</i>	Caroline Pool	WA	Ord	MA08-46	-18.2264	127.7593	1	ABTC 13333	WAM P.34035-012
C044	<i>Neosilurus pseudospinosus</i>	Trib. of Frank River	WA	Ord	MA08-20	-17.3960	128.2222	3	ABTC 145623-5	WAM P.34035-012
C045	<i>Neosilurus pseudospinosus</i>	Un-named creek	WA	Ord	MA08-21	-17.3867	128.1564	1	ABTC 145642	WAM P.34035-012
C046	<i>Neosilurus pseudospinosus</i>	Spillway Creek	WA	Ord	JG89-m17	-16.0307	128.7812	2	ABTC 71528-9	WAM P.34035-012
C047	<i>Neosilurus pseudospinosus</i>	Bow River	WA	Ord	MH13-40	-16.7939	128.2800	1	1	NTMA 02578
C048	<i>Neosilurus pseudospinosus</i>	Old crossing	NT	Victoria	MH13-31	-15.5841	131.1020	1	2	NTMA 02393
C049	<i>Neosilurus pseudospinosus</i>	Little Fitzmaurice	NT	Fitzmaurice	MH17-24	-15.1112	130.3518	1	1	NTM S.18146-001
C050	<i>Neosilurus pseudospinosus</i>	Flora River	NT	Daly	BP06-01	-14.6682	131.6828	1	1	NTM S.17405-004
C051	<i>Neosilurus pseudospinosus</i>	Fergusson River	NT	Daly	MH12-59	-14.0712	131.9740	2	2	NTM S.17405-004

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TABLE 2. (Continued)

ID	Taxon	Locality	State	River system	Field code	Latitude	Longitude	Tissue code	Vouchers examined
Morphology									
mtDNA									
Allozymes									
C052	<i>Neosilurus manjandi</i> sp. nov.	Chapman River	WA	Pentecost	MH14-17	-16.3179	126.9491	1	1
C053	<i>Neosilurus manjandi</i> sp. nov.	Horse Creek gorge	WA	Pentecost	MH14-10	-16.2526	127.5343	2	2
C054	<i>Neosilurus manjandi</i> sp. nov.	Durack River	WA	Pentecost	BBK14-011	-15.8270	127.3588	2	WAM P.34041-011 & NTM S.17750-001
C055	<i>Neosilurus manjandi</i> sp. nov.	Drysdale River	WA	Drysdale	BH75-A1-3	-15.0437	126.9227	1	WAM P.25404-008
C056	<i>Neosilurus manjandi</i> sp. nov.	Mid-Drysdale River	WA	Drysdale	BH75-A1-9	-15.0046	126.9136	5	WAM P.25410-008
C057	<i>Neosilurus manjandi</i> sp. nov.	Mid-Drysdale River	WA	Drysdale	JSM13-DRM	-15.0061	126.9169	1	NMVA.311775-145
C058	<i>Neosilurus</i> hybrid	Salmond River	WA	Pentecost	BBK14-012	-16.2786	127.6977	1	WAM P.34044.004
C059	<i>Porochilus rendahli</i>	Duck Hole Billabong	WA	Fitzroy	DM02-103	-18.5957	124.7086	1	DM103
C060	<i>Porochilus rendahli</i>	Flying Fox Creek	WA	Ord	DB06-A14	-16.5730	128.3410	1	NTM 416
C061	<i>Plotosus lineatus</i>	Arafura Sea	NT	NA	TG15-01	-10.3500	132.7500	1	NTM A 03726
Totals						42	28	55	

TABLE 3. Allozyme profiles for the seven primary *Neosilurus* taxa and F₁ hybrid identified by PCoA. Taxa are designated by their two-letter genus/species code (e.g., NA = *Neosilurus ater*), with distinctive geographic lineages identified by a lower-case epithet indication east, central, or west (e.g., NHw = western lineage of *N. hyrtlii*). For polymorphic loci, the frequencies of the most common allele(s) is/are expressed as percentages and shown as superscripts. Sample sizes in brackets for each taxon. A dash (-) indicates that no profile was able to be assigned at this locus. Invariant loci: *Ak*, *Enol2*, *Est2*, *Got1*, *Mdh1*, *Mdh2*, *Me2*, *Tpi*, and *Ugpp*. Taxon codes as per Table 4.

Locus	NA (14)	NHw (5)	NHc (5)	NHe (5)	NPw (2)	NPe (7)	NSxNPe (1)	NS (3)
<i>Acon1</i>	c ⁸⁶ ,b	c	c	c ⁹⁰ ,a	e	d	bd	b ⁶⁷ ,c
<i>Acon2</i>	a ⁹⁶ ,b	b	b ⁹⁰ ,a	b	-	b	bc	c ⁸³ ,a
<i>Acp1</i>	b	b	b	b	a	b	b	b
<i>Acp2</i>	b	b	b	b ⁹⁰ ,d	c	b	b	a
<i>Acyc</i>	c ⁸⁶ ,b ⁷ ,d	a ⁶⁰ ,d	d	d ⁹⁰ ,c	c	c	cd	d
<i>Ada</i>	c ⁵⁴ ,a ²⁵ ,f ¹⁴ ,d	e	e	e ⁹⁰ ,b	e ⁵⁰ ,f	c	cd	d
<i>Adh</i>	b	a	a ⁸⁰ ,b	a	b	b	b	b
<i>Ald1</i>	b ⁹⁶ ,c	b	b	b	b	b ⁷⁹ ,a	b	b
<i>Ald2</i>	a ⁹³ ,b	a	a	a	a	a	a	a
<i>Ca</i>	c	e	a ⁸⁰ ,c	e	a	b	bd	d
<i>Ck</i>	d	a	a	c ⁹⁰ ,a	d	d	d	d ⁸³ ,b
<i>Enol1</i>	b	b	b	b	b	a	b	b
<i>Est1</i>	d ⁸⁶ ,c ⁷ ,b ⁴ ,e	b	b	d ⁶⁰ ,c ²⁰ ,a ¹⁰ ,e	f	f	cf	c
<i>Fdp</i>	c	c	c ⁹⁰ ,b	c ⁸⁰ ,a	c	c	c	c
<i>Fum</i>	e ⁶⁴ ,b ²⁹ ,a ⁴ ,d	e	e	e ⁸⁰ ,d	f ⁷⁵ ,e	c	ce	e
<i>Gapd1</i>	a	a ⁹⁰ ,b	a	a	a	a	a	a
<i>Gapd2</i>	a ⁶⁹ ,b	a	a	a	a	a	a	a
<i>Glo</i>	c ⁹⁶ ,a	c ⁸⁰ ,b	b	b ⁹⁰ ,c	c	c	bc	b
<i>Got2</i>	c ⁸⁶ ,d ¹⁰ ,b	c	c	c	c ⁷⁵ ,e	a	ac	c
<i>Gp</i>	b	a	a	a ⁸⁰ ,b	a	a	b	b
<i>Gpi1</i>	b ⁶⁸ ,a	a ⁶⁰ ,b	c ⁹⁰ ,b	b ⁹⁰ ,c	d	a ⁷¹ ,c	b	b
<i>Gpi2</i>	a ⁹³ ,b	a	a	a	a	a	a	a
<i>Idh1</i>	b ⁸⁹ ,a ⁷ ,c	b	b	b	b	b	bd	b
<i>Idh2</i>	a	a	a ⁸⁰ ,c	a ⁹⁰ ,c ¹⁰	a	a	a	a ⁶⁷ ,b
<i>Ldh1</i>	b	b ⁹⁰ ,a	b	b	b	b	b	b ⁸³ ,c
<i>Ldh2</i>	a ⁹⁶ ,b	a	a ⁷⁰ ,c	a	a	a	a	a
<i>Mdh3</i>	b ⁸⁹ ,a	b	b	b	b	b	b	b
<i>Me1</i>	c ³⁶ ,d ³⁶ ,e ²¹ ,b	a ⁷⁵ ,c	c ³⁴ ,b ³³ ,a	c ⁹⁰ ,d	a	a	ac	c
<i>Mpi</i>	d ⁹⁶ ,b	b	b ⁵⁰ ,c ⁴⁰ ,a	b	d	d	d	d
<i>Ndpk</i>	a	a	a	a	a	a	ab	b
<i>Np</i>	b	b	c ⁸⁰ ,b	c	b	b ⁹³ ,a	b	b
<i>PepA1</i>	c ⁷⁵ ,a	a	b ⁶⁰ ,a	a ⁷⁰ ,b	a	a	a	a
<i>PepA2</i>	c ⁵⁴ ,a ²⁹ ,d ¹⁰ ,b	d	d	d	c	e	be	b
<i>PepB1</i>	d	a	c	c	b	b	bc	b
<i>PepB2</i>	d ⁸² ,e ¹⁴ ,b	d ⁷⁰ ,b	d ⁸⁰ ,b	a	b ⁵⁰ ,c	d	d	d
<i>PepD</i>	d ⁹⁶ ,e	d	f	c ⁶⁰ ,d ³⁰ ,a	d ⁷⁵ ,b	d	d	d
<i>Pgam</i>	b	b	a	a	b	b	bc	c
<i>6Pgd</i>	a ⁷⁵ ,b ²¹ ,c	c	c	c	c	c	c	c
<i>Pgk</i>	c	c	c	c ⁸⁰ ,a	c	c	bc	b
<i>Pgm</i>	a	d ⁹⁰ ,c	d	d	d ⁷⁵ ,b	d ⁹³ ,e	cd	d
<i>Pk1</i>	a	a ⁷⁰ ,b	a	a	a	a	a	a
<i>Pk2</i>	a	b	b	b	b	b	ab	a

lineages, notably including *Neosilurus* sp. nov., with additional initial indications of sub-structure/cryptic diversity within *N. pseudospinosus* (i.e. Drysdale vs other catchments) and *N. hyrtlili* (same as the three allozyme groups). One anomaly included two fish morphologically identified as *N. pseudospinosus*, but which had *N. ater* mtDNA, possibly an indication of recent hybridisation or older mitochondrial capture within the Isdell River Basin (nuclear data were unavailable to assess different scenarios).

TABLE 4. Pairwise genetic distance measures based on the nuclear genetic data (allozymes) among all *Neosilurus* taxa and F₁ hybrid. Taxa labelled as for Table 3. Lower matrix: number of fixed differences. Upper matrix: unbiased Nei's Distance.

Taxon	NA	NHw	NHc	NHe	NPw	NPe	NSxNPe	NS
<i>Neosilurus ater</i> (NA)	-	0.38	0.48	0.46	0.36	0.37	0.12	0.38
<i>Neosilurus hyrtlili</i> west (NHw)	15	-	0.15	0.17	0.31	0.31	0.17	0.44
<i>Neosilurus hyrtlili</i> central (NHc)	16	5	-	0.12	0.41	0.43	0.23	0.45
<i>Neosilurus hyrtlili</i> east (NHe)	16	6	5	-	0.44	0.46	0.21	0.42
<i>Neosilurus pseudospinosus</i> Drysdale (NPw)	14	12	13	17	-	0.22	0.15	0.43
<i>Neosilurus pseudospinosus</i> Ord (NPe)	15	13	15	19	11	-	0.05	0.47
<i>Neosilurus</i> hybrid (NSxNPe)	8	10	11	10	9	3	-	0
<i>Neosilurus manjandi</i> sp. nov. (NS)	14	15	16	15	17	19	1	-

TABLE 5. Summary of mtDNA genetic data (cytb) mean between species pairwise p-distance values for Kimberley plotosid catfishes and reference material/outgroups. Values on the diagonal represent mean within species p-distance values, with n/c representing species with only a single sequence.

	<i>N. hyrtlili</i>	<i>N. manjandi</i>	<i>N. ater</i>	<i>N. pseudospinosus</i>	<i>N. mollespiculum</i>	<i>P. rendahli</i>	<i>A. dahli</i>	<i>Pl. lineatus</i>
	sp. nov.							
<i>Neosilurus hyrtlili</i>	0.019							
<i>Neosilurus manjandi</i> sp. nov.	0.097	0.001						
<i>Neosilurus ater</i>	0.102	0.075	0.011					
<i>Neosilurus pseudospinosus</i>	0.107	0.087	0.091	0.021				
<i>Neosilurus mollespiculum</i>	0.108	0.089	0.089	0.081	n/c			
<i>Porochilus rendahli</i>	0.161	0.155	0.165	0.165	0.167	0.022		
<i>Anodontiglanis dahli</i>	0.181	0.160	0.165	0.163	0.169	0.183	0.042	
<i>Plotosus lineatus</i>	0.216	0.209	0.204	0.207	0.211	0.210	0.205	n/c

Morphology

An analysis of seven meristic counts for individuals representative of Kimberley *Neosilurus* taxa showed clear separation in ordination space (PCA), as distinct groups matching the existing taxonomy, plus recognition of *Neosilurus* sp. nov. (Fig. 3b). The new species was well separated from both *N. ater* and *N. hyrtlili* on dimension 1, correlating with a higher number of second dorsal fin rays (correlation value 0.89), more precaudal vertebrae (0.73) and fewer anal fin rays (-0.71), and separated from *N. pseudospinosus* on dimension 2 based on fewer caudal vertebrae (0.88). The F₁ hybrid was closer to one parental taxon in ordination space (the new species), making it difficult to identify without nuclear genetic data (the hybrid had precaudal vertebrae number characteristic of *N. pseudospinosus*, but qualitatively possessed hard spines similar to *Neosilurus* sp. nov.). Regional variation was also explored within several species. There was some variation noted between fish representing different catchments of *Neosilurus* sp. nov., with those from the Drysdale showing a greater level of heterogeneity than those from

the Pentecost, but with general overlap not suggestive of two distinct clusters (no genetic material was available from the Drysdale as a comparative framework). No catchment/regional variation was noted in *N. hyrtlii* and *N. pseudospinosus* at the level of investigation undertaken; clearly a thorough investigation would require greater replication of voucher material (or new collections for sample availability). A summary of the raw representative meristic data for Kimberley *Neosilurus* samples is shown in Tables 6–7, and discussed further in the taxonomic treatment below.

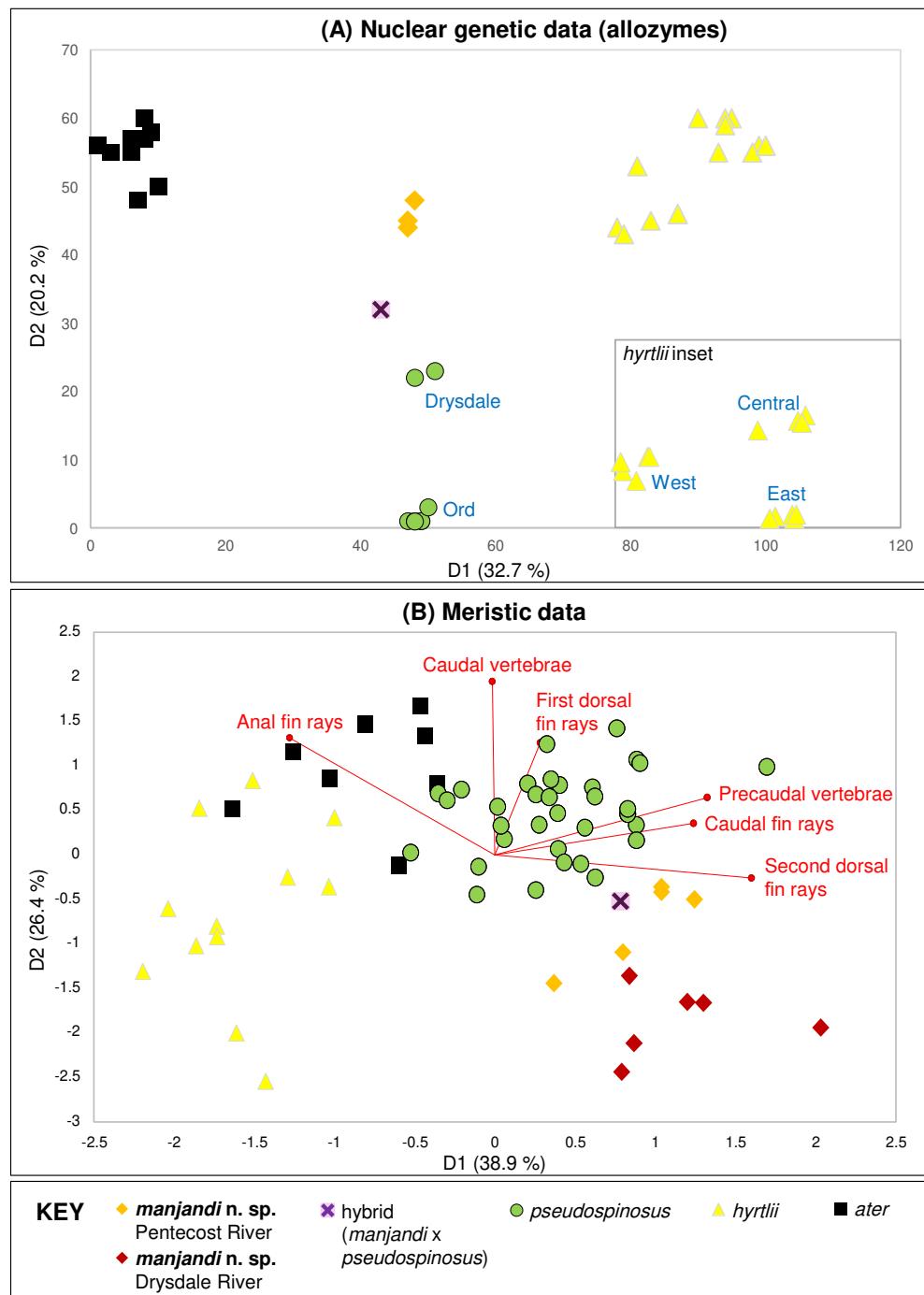


FIGURE 3. Ordination analyses of individual data for Kimberley *Neosilurus*: (A) PCoA of nuclear genetic data (51 allozyme loci), the inset shows a follow-up ordination of the *N. hyrtlii* cluster suggestive of three geographic groups namely western (lower Fitzroy, Lennard and Isdell catchments), central (upper Fitzroy, Prince Regent and Drysdale catchments) and eastern (Durack, Ord and Victoria catchments); and (B) PCA of representative meristic data (7 characters rapidly obtainable from radiographs) including correlation vectors.

TABLE 6. Summary of representative meristic data for Kimberley *Neosilurus* species, with data for holotype of *Neosilurus manjandi* sp. nov. marked (*).

Precaudal vertebrae	7	8	9	10							
<i>ater</i>	1	8	2								
<i>hyrtlii</i>	2	10									
<i>pseudospinosus</i>		11	4								
hybrid		1									
<i>manjandi</i> sp. nov. Drysdale	1	5									
<i>manjandi</i> sp. nov.											
Pentecost		5*									
Caudal vertebrae	34	35	36	37	38	39	40	41	42	43	44
<i>ater</i>						1		1		8	1
<i>hyrtlii</i>	1		1	2	2	2	3		1		
<i>pseudospinosus</i>						1	7	2		5	
hybrid									1		
<i>manjandi</i> sp. nov. Drysdale		2	1	2	1						
<i>manjandi</i> sp. nov.											
Pentecost						5*					
Second dorsal fin rays	20–22	23–25	26–28	29–31	32–34	35–37	38–40	41–43	44–46	47–49	50–52
<i>ater</i>			1	3	4	3					
<i>hyrtlii</i>	1	10	1								
<i>pseudospinosus</i>						7	6	2			
hybrid								1			
<i>manjandi</i> sp. nov. Drysdale									3	2	1
<i>manjandi</i> sp. nov.									4*	1	
Pentecost											
Anal fin rays	65–67	68–70	71–73	74–76	77–79	80–82	83–85	86–88	89–91		
<i>ater</i>					1	3	2	3	2		
<i>hyrtlii</i>					2	3	6	1			
<i>pseudospinosus</i>			1	3	7	3			1		
hybrid		1									
<i>manjandi</i> sp. nov. Drysdale	2	3		1							
<i>manjandi</i> sp. nov.											
Pentecost			4*	1							
Caudal fin rays	7	8	9	10							
<i>ater</i>		2	9								
<i>hyrtlii</i>	1	8	3								
<i>pseudospinosus</i>		4	10	1							
hybrid			1								
<i>manjandi</i> sp. nov. Drysdale			5	1							
<i>manjandi</i> sp. nov.											
Pentecost	1	4*									

TABLE 7. Morphological data for *Neosilurus manjandi* sp. nov.

Character	Holotype	Average	Min	Max	n
Total length	103.7	104.2	62.0	173.6	11
Standard length	91.3	95.8	60.3	152.5	10
<i>Morphometric%</i>					
Body depth/SL	16	16	15	17	10
Head length/SL	22	22	22	23	10
Head width/HL	68	65	78	70	10
Head height/HL	61	58	53	65	10
Snout length/HL	41	43	40	46	10
Eye diameter/HL	22	20	19	22	10
Interorbital width/HL	33	34	33	36	10
Nasal barbel length/HL	57	59	49	84	10
Maxillary barbel length/HL	80	74	60	96	10
Outer mental barbel length/HL	77	71	57	82	10
Inner mental barbel length/HL	44	50	44	73	10
Caudodorsal fin length/SL	28	29	28	31	10
First dorsal fin height/HL	79	81	76	87	10
Pectoral fin length/HL	66	68	63	75	10
Pelvic fin length/HL	50	48	41	57	10
Predorsal length/SL	27	28	27	29	10
Anal fin length/SL	58	57	56	58	10
Preanal length/SL	43	43	41	45	10
<i>Meristic counts</i>					
Precaudal vertebrae	9	9	8	9	11
Caudal vertebrae	39	39	35	39	11
Total vertebrae	48	48	44	48	11
First dorsal fin rays	5	4	4	5	11
Second dorsal fin rays	45	46	44	52	11
Anal fin rays	72	72	65	74	11
Caudal fin rays	9	9	8	10	11
Total caudodorsal fin elements	126	128	119	130	11
Pectoral fin rays	13	12	11	13	11
Pelvic fin rays	14	14	12	14	11
Branchiostegal rays	8	8	8	9	11
Gill rakers upper	8	7	7	8	9
Gill rakers lower	20	20	19	21	9
Total rakers	28	28	26	29	9

Description

Neosilurus manjandi sp. nov.

urn:lsid:zoobank.org:act:8DCA0F5F-1C6A-4089-B735-2560443B72C6

Sandstone Catfish

Ngarinyin name: Walaman jirri (generic sharp-spined plotosid)

Figs 1–7, Tables 2–7

Neosilurus (Tandanus) ater nec Perugia: Hutchins, 1977: 103 (in part; two specimen lots).

Neosilurus ater nec Perugia: Moore and Hammer, 2015: 68 (in part; four specimen lots and one hybrid specimen).

Material Examined (all Western Australia). Holotype: WAM P.34043-011, 91.3 mm SL, small falls upstream at Jack's Waterhole, Durack River, Pentecost River system, G. Moore, M. Hammer & P. Jackson, back-pack electrofisher, 3 June 2014. Paratypes: NTM S.17750-001 (aquarium image), 126.5 mm SL, data as per holotype; WAM P.34041-011 (tissue A 03241), 102.9 mm SL, downstream of Scotty-Salmon Gorge, Chapman River, Pentecost River system, G. Moore & M. Hammer, back-pack electrofisher, 3 June 2014; WAM P.34035-011 (tissues A 03129–130), 2 specimens 83.7–95.5 mm SL, below cliff, Horse Creek, Pentecost River system, G. Moore, M. Hammer & L. Smith, back-pack electrofisher, 28 May 2014; NMV A.31775-145, 173.6 mm SL, mid-section Drysdale River, J. Shelley & M. Le Feuvre, 27 January 2013; WAM P.25404-008, 102.4 mm SL, rapids mid-section Drysdale River, B. Hutchins, rotenone, 4 August 1975; WAM P.25410.008, 4 specimens 62.0–121.1 mm SL (one cleared and stained), mid-section Drysdale River, B. Hutchins, rotenone, 7 August 1975. Comparative material: see Table 2.

Diagnosis. A small plotsid with a moderately extended caudodorsal fin (originating just posterior to mid-point of body), small first dorsal fin, depressed head, hard dorsal and pectoral spines, first dorsal I,4–5, second dorsal 44–52, pre-caudal vertebrae 8–9 and caudal vertebrae 35–39.

Description. Based on 11 specimens, 62.0–173.6 mm SL, with a full summary of data provided in Table 7. Counts and measurements of holotype are indicated with an asterisk (*) within range.

Body eel-like, moderately long and slender, laterally compressed, not tapering greatly posteriorly; minute papillae densely covering skin on head and body (development varying across individuals); first dorsal fin relatively short, its tip rounded, originating behind vertical through pelvic fin origin; pectoral fins inserted just behind opercular margin; tips of pectoral fins slightly pointed to well rounded; posterior edges of pectoral fin spines with few but large serrations on inner half; caudodorsal fin originating moderately far forward, just posterior to mid-point of body or above middle of anal fin.

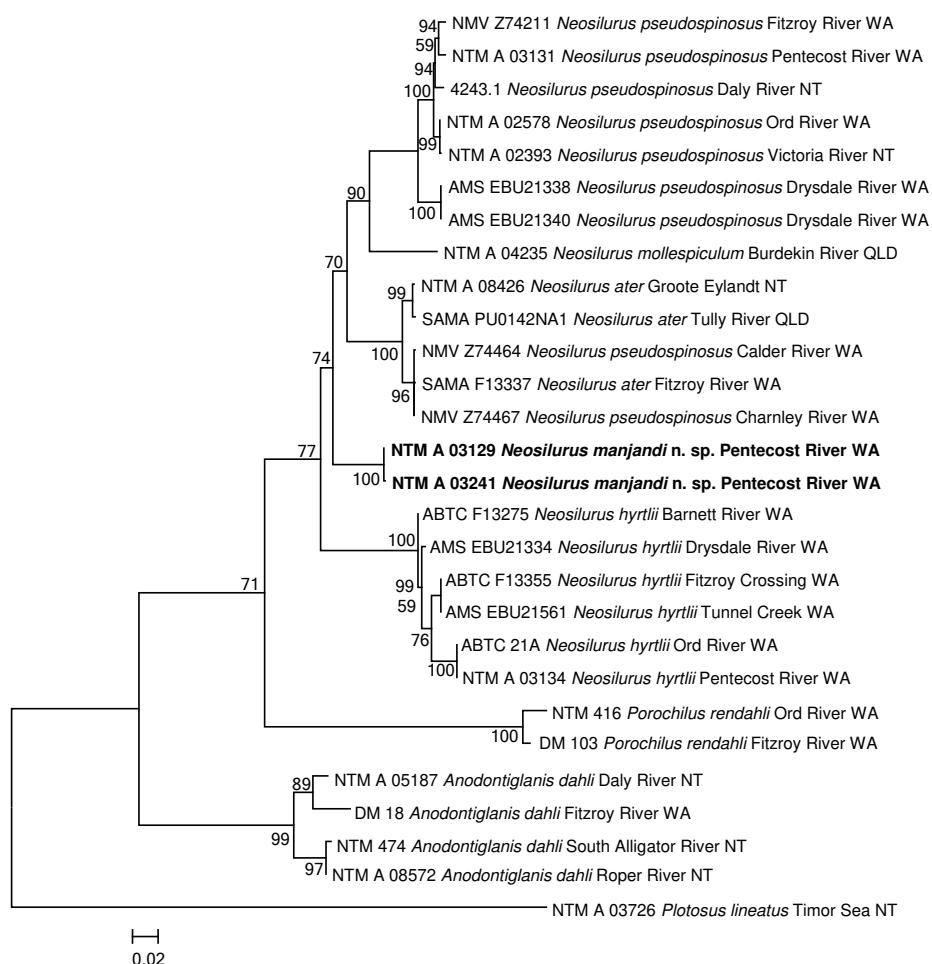


FIGURE 4. Mitochondrial DNA phylogenetic tree for Kimberley plotosid catfishes and representative comparative material, plus marine outgroup; ML tree for 1138 bp of cytochrome oxidase *b*, sample details provided in Table 2.

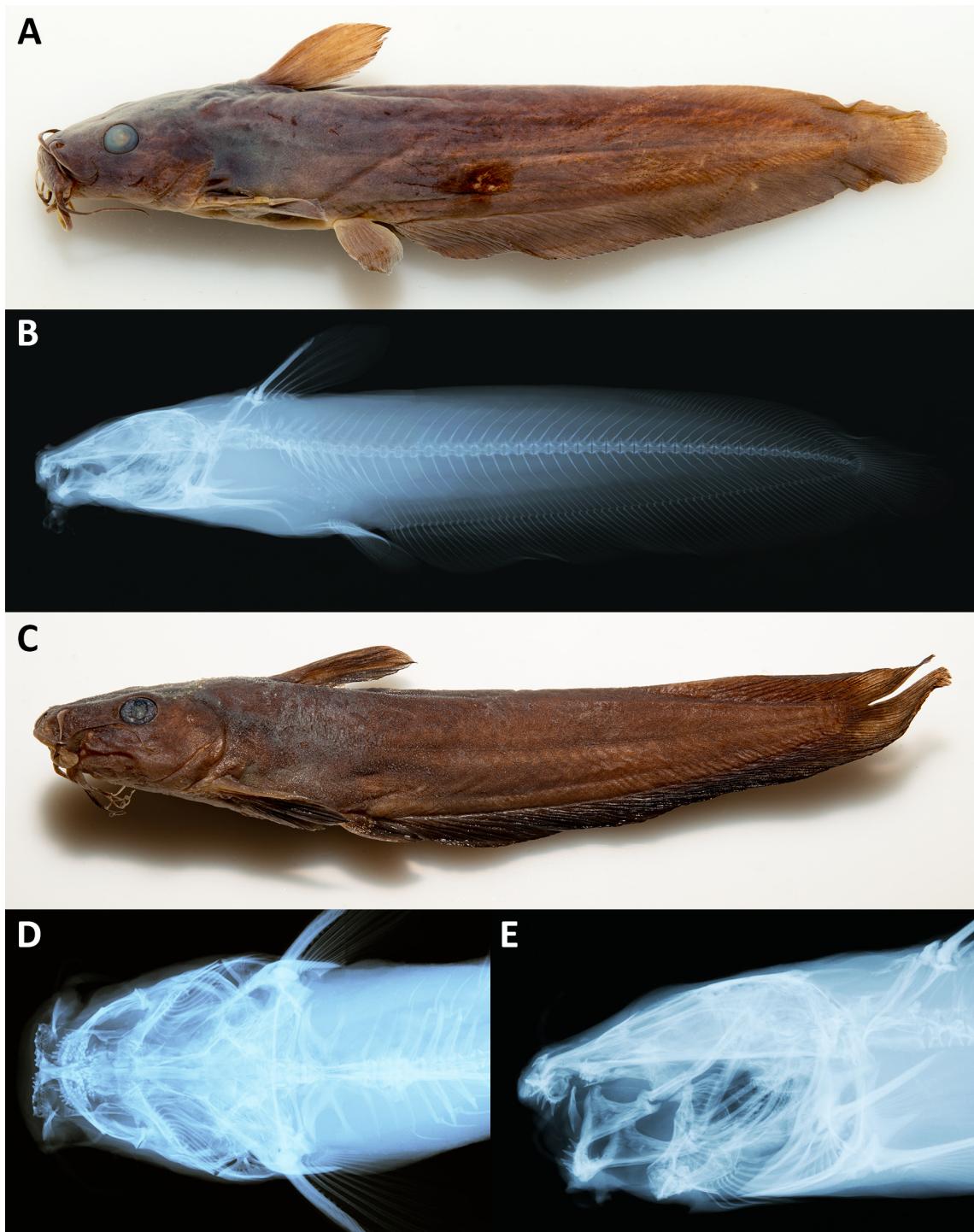


FIGURE 5. Specimen images of *Neosilurus manjandi* sp. nov.: (A + B) lateral view of specimen and radiograph of holotype WAM P.34043-011, 91.3 mm SL, Jack's Waterhole, Durack River, Pentecost River Catchment; (C) specimen image of paratype NMV A.31775-145, 173.6 mm SL, mid-section Drysdale River; (D) dorsal head radiograph of paratype NMV A.31775-145 showing teeth patterns; and (E) lateral head radiograph of paratype NTM S.17750-001, 126.5 mm SL, data as per holotype, including details of dentition.

Head robust, moderately depressed dorsoventrally, its width similar to height and 66% of head length; dorsal profile of head slightly convex and relatively flat; snout rounded in lateral view, its length about 40% of head length; mouth subterminal; lips fleshy; anterior naris a short tube above upper lip; posterior naris with ovate opening and positioned directly behind nasal barbel; eyes moderately sized, on anterior half of head and dorso-laterally positioned; all barbels relatively long, reaching beyond eye (nasal and inner mental) or well beyond eye close to

base of gill opening (maxillary and outer mental); dermal fold on chin moderately well developed, forming pit midway between lower lip and ventral gill opening; branchiostegal membranes fused within fleshy gill cover and free from isthmus; oral dentition composed of long slender conical teeth in 2–3 rows on upper and lower jaws with outer teeth largest, and a patch of 5 mainly stout conical teeth on palate.



FIGURE 6. Images of *Neosilurus manjandi* sp. nov. and its sandstone habitat at type locality on Nyaliga Country, small waterfall upstream of Jack's Waterhole, Durack River, Pentecost River catchment, Australia: (A) live image of fish in an aquarium (NTM S.17750-001 paratype, 126.5 mm SL: photo by Nathan Litjens); (B) flowing microhabitat; and (C) sandstone mesohabitat.

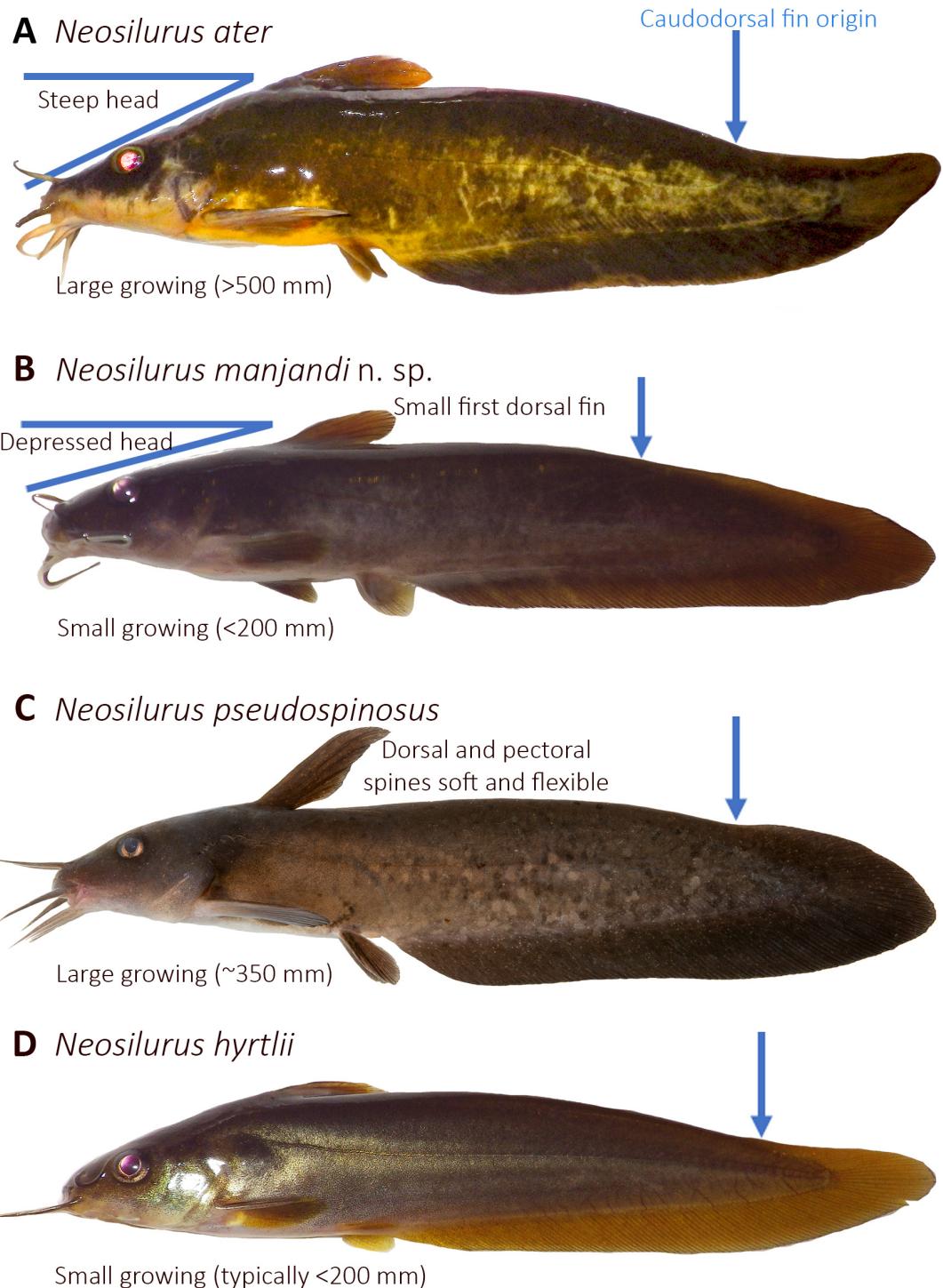


FIGURE 7. Comparative photos of Kimberley *Neosilurus* catfishes showing key identification features. Specimen details: *Neosilurus ater* Moonshine Gorge, Pentecost River, NTM S.17671-016 (tissue A 02440); *Neosilurus manjandi* sp. nov. Horse Creek, Pentecost River, WAM P.34035-011 (tissue A 03130); *Neosilurus pseudospinosus* Little Fitzmaurice River, NTM S.18146-003, and *Neosilurus hyrtlji* Horse Creek, WAM P.34035-010 (tissue A 03134).

Precaudal vertebrae 8–9*; caudal vertebrae 35–39*; total vertebrae 44–48*; first dorsal fin rays I,4 or 5*; second dorsal fin rays 44–52 (45*); anal fin rays 65–74 (72*); caudal fin rays 8–10 (9*); total caudodorsal fin elements 119–130 (126*); pectoral fin rays I,11–13*; pelvic fin rays 12–14*; branchiostegal rays 8*–9; gill rakers on first arch 7–8* upper plus 19–21 (20*) lower; total rakers 26–29 (28*).

Body depth 15–17 (16*)% of SL; head length 22*–23% of SL; head width 65–78 (68*)% of HL; head height 53–65 (61*)% of HL; snout length 40–46 (41*)% of HL; eye diameter 19–22*% of HL; interorbital width 33*–36% of HL; nasal barbel length 49–84 (57*)% of HL; maxillary barbel length 60–96 (80*)% of HL; outer mental barbel length 57–82 (77*)% of HL; inner mental barbel length 44*–73% of HL; caudodorsal length 28*–31% of SL; first dorsal fin height 76–87 (79*)% of HL; pectoral fin length 63–75 (66*)% of HL; pelvic fin length 41–57 (50*)% of HL; predorsal length 27*–29% of SL; anal fin length 56–58*% of SL; and preanal length 41–45 (43*)% of SL.

Colouration of preserved material. Uniform dark to light brown, lighter on ventral surface of abdomen (Fig. 5).

Colouration of fresh material. Variable, from uniform dark brown to yellowish brown, with some speckling, and abdomen paler yellow-grey. Fins brown, but sometimes with yellowish hue, especially when freshly caught and illuminated with sunlight (Figs 6–7).

Comparisons. The new species is superficially similar to three other co-occurring *Neosilurus* in the Kimberley, which can all display a generally darker brown body colour in the right circumstances (see visual guide to species in Fig. 7). However, the dorsal extent of the caudodorsal fin (to about half length of body, correlating with high second dorsal fin rays = 44–52) is greater in comparison to all other species in the region (1/3 or less the length of anal fin, <43 rays), and most contrasting to the very short extent observed in *N. hyrtlii* (<30 rays). The small first dorsal fin, gently sloping head and fewer anal fin rays (<75) are a further distinction when compared with the larger growing, steep headed *N. ater*. The stout dorsal and pectoral spines that project with a sharp tip contrast with the soft flexible spines of *N. pseudospinosus*, which can be further separated from the new species by its higher number of caudal vertebrae (typically 40 or more vs 35–39).

Distribution. Poorly known, but so far recorded from two locations representing (a) three streams in the Pentecost River system, and (b) a small section of the mid-Drysdale River main channel, and likely to be more widespread in suitable habitats (Fig. 1). To place this geographic range in context referencing IUCN Red List assessment criteria, it is well less than the 5000 km² extent of occurrence threshold of Criterion B (Geographic Distribution) for being considered Endangered (IUCN, 2024), in conjunction with current and future threats (see discussion section below).

Ecology. Recorded in rocky stream sections including gorge and constrained valleys, in flowing stream or riffle microhabitats (dry season conditions), and likely to occupy larger refuge pools nearby (Fig. 6). The presence of relatively large teeth may be an indication of a specialised predatory diet; otherwise, all aspects of biology remain to be determined.

Etymology. The species epithet is derived from *manjan di*, from the language of the Ngarinyin people that literally means sandstone, in reference to the species habitat. The name was chosen in collaboration with the Nyaliga Aboriginal Corporation on whose Country the holotype specimen (type locality) of this species was collected.

Discussion

The Kimberley region is a biodiversity hotspot including for fishes, and remains a priority region for taxonomic studies (Morgan *et al.*, 2011; Shelley *et al.*, 2018a; Shelley *et al.*, 2019). Plotosid catfishes is a prominent regional group in terms of species representation and cultural importance. Additionally, some species with more specialised habitat requirements or restricted ranges potentially serve as key environmental indicators and targets for land management, water resource planning and conservation (Anderson and Maldonado-Ocampo, 2011; Leonard *et al.*, 2013; Shelley *et al.*, 2018a; Le Feuvre *et al.*, 2021). This is reinforced by the description of a new range-restricted freshwater catfish from specific sandstone flowing stream habitats.

Further research is required to better define the range, distribution and ecology of the new species to underpin future conservation assessment and action. This extends to trialing different survey techniques such as fyke netting larger pools, eDNA approaches and targeting particular microhabitats, using the refined identification search image from the visual guide developed herein, to confirm if the species is genuinely rare.

The habitats of the Kimberley are recognised as being in relatively good condition, however a range of current and future threats are known that may impact the conservation of *N. manjandi* (Morrongiello *et al.*, 2011; Shelley *et al.*, 2018a). Given the wet/dry nature of the Kimberley climate, driven by seasonal and inter-annual variation in rainfall, extended hot dry periods can lead to major contraction in water availability, including riffle and shallow

pool drying. Maintaining refuge pools is a likely key conservation consideration in the face of current land use (e.g. grazing of riparian zones), any future catchment modification, and climate change.

While the current study had a specific focus on examining a suspected distinctive morphotype, the assessment was placed within a patchwork of different data types and samples to form an overview of species boundaries in the Kimberley plotosid fauna. Our preliminary data highlighted potential cryptic diversity within most of the described “species”. Further focused systematic sampling is clearly required to explore this additional biodiversity. Importantly, this sampling can only be considered comprehensive if it moves beyond simple mtDNA barcoding, which has demonstrably failed to fully assign some individuals to their correct taxa in this study and has proved unreliable in many freshwater fish studies (e.g. Adams *et al.*, 2014; Shelley *et al.*, 2018b). Instead, the focus should be on next generation sequencing and/or multi-locus nuclear genetic markers (i.e. best practice for species boundaries, and to specifically assess hybridisation and introgression), with comprehensive spatial coverage to help more fully evaluate these patterns (Unmack *et al.*, 2022).

As a focus for future taxonomic assessment, distinct lineages were noted within *A. dahli* (Kimberley vs Daly vs northern Australia), *N. hyrtlii* (western vs central vs eastern Kimberley, and clear need for a national overview), and *N. pseudospinosus* (Drysdale River vs rest of range). Indeed, some morphological variation for *N. manjandi* from the two different river basins also warrants dedicated effort to collect genetic tissues from the remote mid-Drysdale to complete the comparison, which either way should be managed as separate evolutionary distinct units.

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