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A new species of burrowing frog, *Neobatrachus* (Anura: Myobatrachidae), from the eastern wheatbelt of Western Australia.

J.D. Roberts*, M. Mahony†, P. Kendrick*§ and C.M. Majors*

Abstract

A new species of *Neobatrachus* is described from the wheatbelt of Western Australia. This species has a diploid karyotype and can be distinguished from congeneric species by morphology and call.

Introduction

The delineation and identification of species of *Neobatrachus* has often proved difficult using morphological features alone. The analysis of chromosomes and mating call, however, have facilitated the resolution of species boundaries in this genus (Roberts 1978; Mahony and Robinson 1980; Mahony and Roberts 1986). Having defined species by call and/or karyotype it has often been possible to find consistent morphological differences between species (e.g. Mahony and Roberts 1986).

During field work in the eastern wheatbelt from 1983 to the present we have obtained, from several localities, specimens of a diploid *Neobatrachus* species which differs in call structure and appearance from *N. pelobatoides:* the only other diploid species we have observed in this area. These specimens are herein referred to a new species.

Materials and Methods

Frogs were collected and calls recorded during field work from 1983 to 1989. Call recording and analysis techniques and karyotype preparation follow Mahony & Roberts (1986). Genetic differentation of species was assessed using gel electrophoresis of soluble enzymes and proteins (Richardson *et al.* 1986); detailed methods follow Barendse (1984). Electrophoretic data were collected from five specimens of the new species (four from 20.5 km W Jerramungup; one from 4.2 km N Hopetoun) and, 31 specimens of *N. pelobatoides* (eight from 8.9 km SE of Beverley, 19 from 20.5 km W Jerramungup, and four from 4.2 km N Hopetoun). Because the new species is diploid, it was not necessary to include the tetraploid species *N. kunapalari* in the electrophoretic comparisons. Twenty-five presumptive gene loci were scored, including four non-enzymic general protein bands (Appendix 1).

Morphological differentiation was assessed by the same set of morphometric variables as reported by Mahony & Roberts (1986) with the addition of naris-snout (N-S, defined in Roberts, Wardell-Johnson and Barendse 1990), anterior interorbital

*Department of Zoology, University of Western Australia, Nedlands, W.A. 6009.
†Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, S.A. 5000.
§Department of Conservation and Land Management, P.O. Box 835, Karratha, W.A. 6714.

distance (ant I-O, equivalent to inter-orbital distance of Roberts, Wardell-Johnson and Barendse 1990), posterior interorbital distance (post. I-O, distance between posterior corners of eyes) and width and height of tympanum (tymp. W and tymp. H, skin was not removed). Morphometric data were collected from 14-16 male specimens of each species as follows (all numbers refer to specimens in the W.A. Museum (WAM) collection, Perth. The letter prefixing registration numbers is an institutional identification): the new species, the type series; *N. pelobatoides,* R101156, R101158-59, R101162, R101164, R101166-67, R101170, R101177-78, R33365, R39535, R94426, R94428, R94429 and *N. kunapalarai,* R101187-88, R101197-99, R101201, R101204, R101207-8, R101210-11, R101213, R101214. Metric variables were analysed by one-way analysis of variance using Statview 512+ software on a Macintosh Plus personal computer.

Distribution data are based on WAM records and our own field recordings or observations of calling males.

Systematics

Neobatrachus albipes sp. nov.

Figures 1 - 3, 5.

Holotype

R101178, adult male, 37.2 mm SV, collected 4.2 km N Hopetoun, W.A., (33°54'S, 120°08'E) by J.D. Roberts and P. Kendrick, 3 May, 1988. Calls recorded as #3 on JDR Tapes 79 & 80. Tapes housed in the Department of Zoology, University of Western Australia.

Paratypes

Fourteen males: R36291, 41.6 km E Southern Cross, W.H. Butler, February 16, 1970; R39841, Greenshield Soak, near Lake Magenta, L.A. Smith *et al.* May 4, 1971; R49800, Dongolocking Nature Reserve, WAM Biological Survey, May 15, 1975; R52546 & R52550, Badjaling Nature Reserve, 11 km E Quairading, WAM Survey, May 6, 1975; R65144, 0.6 km W Lake Cronin, WAM Biological Survey, September 19, 1978; R93378, Junana Rock, Cape Arid National Park, L.A. Smith & R.A. Johnstone, November 3, 1985; R94796, Lake Magenta Nature Reserve, M.S. Graham, July 23, 1986; R96779, Fitzgerald River National Park, B. Maryan *et al.*, July 23, 1986; R101179, 20.5 km E Jerramungup, J.D. Roberts & P. Kendrick, May 2, 1988; R101185, same site as R101179, C.M. Majors, May 24, 1989; R101183-84, turn off to Pallarup Rocks, 44.6 km NE of Ravensthorpe, J.D. Roberts & P. Kendrick, May 3, 1988; R101186, Yoting, rubbish dump at turn off to Kellerberrin on Quairading-Bruce Rock road, J.D. Roberts, C.M. Majors, A. Savage & B. Murray, May 12, 1988.

One female: WAMR101180, 20.5 km E Jerramungup, May 2, 1988, J.D. Roberts & P. Kendrick.

Diagnosis

Moderately sized frog (adult males 33.2 to 45.4 mm S-V; female 35.3 mm S-V), light brown or grey with diffuse darker markings. Mating call a series of 33 to 40 pulses with a very high pulse repetition rate. Karyotype diploid. Metatarsal tubercle unpigmented or lightly pigmented. Upper surface of foot white, distinct light bar between eyes.

Description of holotype

Head wider than long (HW/ HL 1.18), slopes down from eye to naris. Snout rounded when viewed dorsally, almost flat in profile. Lines from eye to naris and from snout tip to naris intersect on prominent naris at angle of about 110° so snout appears almost square in profile. Nares directed upwards and slightly lateral. Eye large (EL/ HL 0.463) and

prominent. Tympanum higher than broad (TH/TW 1.23) and obvious despite being covered by skin. Fingers slender, short, unwebbed (3>1>2>4). Prominent subarticular tubercle at first joint on all fingers. Prominent tubercle between fingers 1-2 and 2-3 but not between 3-4. Large, rounded, flattened inner metacarpal tubercle, elongate outer metacarpal tubercle. Nuptial pads on first two fingers from base to distal joint. Maxillary teeth present, vomerine teeth in short medially separated series between choanae, medial gap about equal to gap between lateral end of vomerine tooth row and adjacent choana. Distinct parotoid glands from behind tympanum extending medially almost to middle of back. Toes webbed. Webbing extends to end on first, second and third toes, to second joint on fourth and similar on fifth on right foot. Left foot is abnormal with only four toes obvious. Prominent, white, shovel-shaped inner metatarsal tubercle. No outer metatarsal tubercle. Weakly developed, subarticular tubercles on toes three, four only. Limbs moderate (T/SV 0.37), foot long (FL/T 1.76). Toes slender, 4>3>5>2>1. Colour in preservative: dorsum brown with irregular darker markings with poorly defined edges. Broad, lighter "V" shaped mark with well defined posterior margin between and extending onto skin above eyes. Similar, broad, darker "V" shaped bar on posterior portion of head and extending onto skin above eyes. Ventral surface of body, legs and upper-arms creamy-white, similar colour on inner margin of feet, anterior side of fore-arms. Plantar and palmar areas dark brown. Anterior and lateral margins of

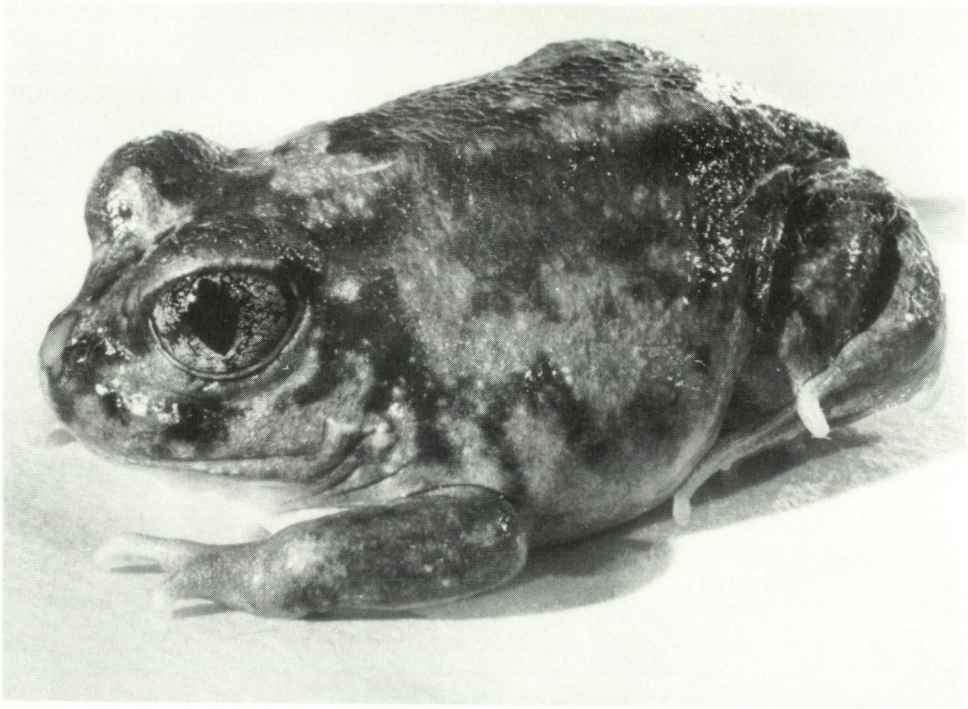

Figure 1. Holotype in life, photographed by S. Hopwood.

submandibular skin suffused with dark grey-brown. Upper surface of foot and toes white with the ankle skin clear making it appear dark brown from the underlying muscle. In life, colour varied with the colour of background. On dark backgrounds, colour similar to above. On light backgrounds, general colour was grey to a light green-yellow, particularly on the flanks and posterior half of body, with darker markings.

Measurements of holotype (mm): snout-vent 37.2, head width 15.4, head length 13.0, eye-naris 2.7, inter-naris 2.7, naris-snout 1.3, inter-orbital (anterior) 6.6, inter-orbital (posterior) 12.0, tibia 13.6, foot 24.0, tympanum width 3.2, tympanum height 4.0.

Figure 1 shows holotype in life.

Variation

Paratypes showed the following minor differences from holotype: R10118 has mid-dorsal stripe extending from level of tympanum to cloaca. Mid-dorsal stripe also present on R52550. Parotoid glands more obvious in some, lighter brown-yellow in preservative. Distinctness of light bar between eyes varies; no bar as such in R101184 but still a distinct light spot between eyes. In R52550, R65144 and R93378 metatarsal tubercle faintly edged with light brown. R49800 and R39481 have nuptial pad as a thin line on the third finger as well as on first and second fingers.

Mating call

Detailed data and an oscillograph are given for call of holotype (Table 1, Figure 2). Mating calls have been recorded at several sites (Figure 3) and detailed analyses are given in Table 1. Call is short, a series of 36 to 40 pulses (mean 36) rapidly repeated (32 pulses s^{-1} at 11.4°C), with an average dominant frequency of 1028 Hz. Pulse repetition rate and call duration vary with temperature but other variables do not (Roberts, unpublished data). The difference between the dominant frequency for the call of the holotype (1266 Hz) and the frogs recorded near Bruce Rock (mean 1028) is attributed to body size differences. The lowest dominant frequency at Bruce Rock was from a frog with a S-V length 5mm longer than the holotype. Body size is negatively correlated with dominant frequency in *Neobatrachus pictus* (Roberts 1978).

Table 1. Call structures in *Neobatrachus* from Western Australia. Values given are means ± standard error. Variables are: sample size (n), pulse number, pulse rate (pulses s^{-1}), pulse duration (ms), call duration (s), dominant frequency (Hz), pulse rise time as a % of pulse duration and water temperature (°C).

Species	n	Pulse number	Pulse rate	Pulse duration	Call duration	Dominant freq.	Rise time	Water temp.
N. albipes - holotype	1	39.0	52.5	9.4	0.74	1266	27.1	18.4
N. albipes[1]	6	35.6±1.0	31.8±.4	8.8±.5	1.11±.05	1028±17	29.1±1.5	11.4±.6
N. kunapalari[1]	8	19.9±1.4	12.4±.8	18.8±.8	1.59±.15	1101±31	19.3±1.2	11.7±.5
N. pelobatoides[2]	3	59.8±7.6	18.9±1.6	11.2±.9	3.23±.32	816±28	48.3±3.8	12.3±.2

[1]4.3 km NW of Bruce Rock, on Doodlakine Road, 24 and 26, May, 1989

[2]Data from Table 2 of Mahony & Roberts 1986

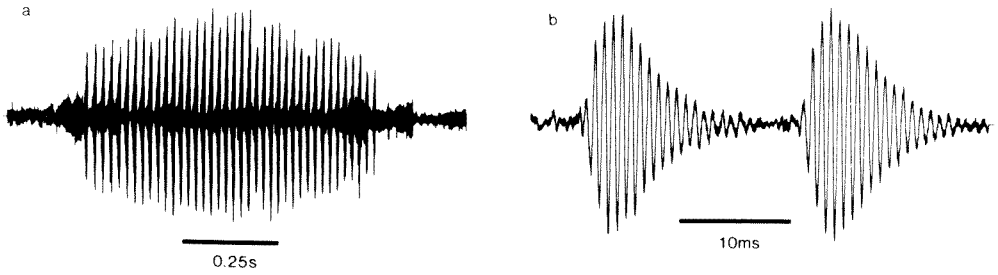

Figure 2. Oscillographs of call of the holotype: a) complete call b) detail of pulse structure showing two complete pulses.

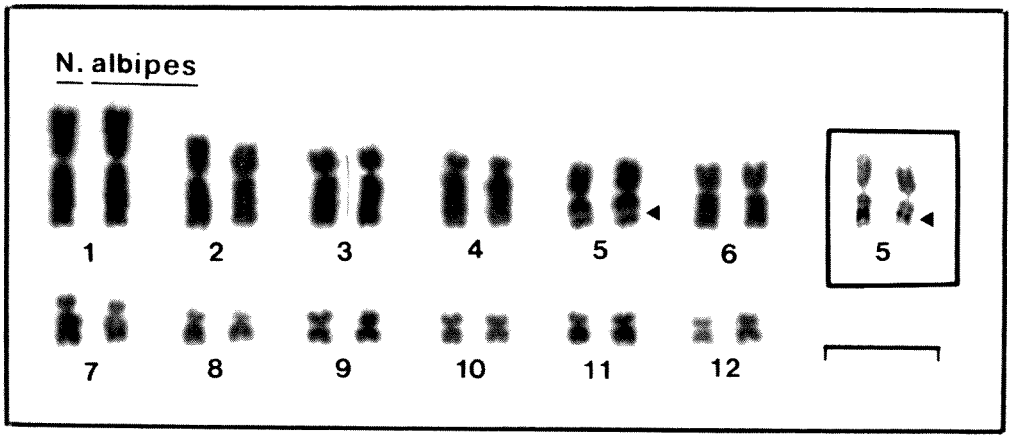

Figure 3. Karyotype of *N. albipes*. Inset: silver stained NOR. Scale bar 10μm.

Breeding biology

Males have been heard calling after summer rains (January 25, 1982, Narembeen, Kondinin), but most activity has been observed after autumn rains (May 1, 1988, W of Coolgardie; May 2-3, 1988 Jerramungup, Hopetoun area; May 12, 1988, Yoting; May 24-26 1989, Bruce Rock, Jerramungup, Ongerup, Lake Cronin) and early winter rain (12-16 June, 1989 Corabbin, Bodallin, Moorine Rock). Calls have also been recorded by A. Chapman at Ravensthorpe after a thunderstorm on March 3, 1983 and by D. Cale on the Chester Pass Road, 28 km east of Mount Barker, October 15, 1985. Breeding is typical of *Neobatrachus:* explosive activity for one or two nights after heavy rain. Males generally call from covered sites, under bushes or other flooded vegetation, and often in deep water (30-50 cm deep or more at sites near Hopetoun and at Bodallin). Males do not seem to move about as much as observed in choruses of *N. pictus* (Roberts 1978) and *N. kunapalari* (Mahony & Roberts 1986) but this has not been quantified. Amplexus is inguinal but egg deposition has not been observed. Males have been heard calling at water temperatures from 9.8°C to 19.8°C.

Karyotype

Neobatrachus albipes is diploid (2n = 24, Figure 3). As such it is readily distinguished from tetraploid congeners: *N. kunapalari, N. centralis* and *N. aquilonius*. The relative lengths and centromere positions of the chromosomes are very similar to those of both diploid and tetraploid species of this genus (see Mahony and Robinson 1980; Mahony and Roberts 1986). *Neobatrachus albipes* cannot be readily distinguished from diploid congeners with the exception of *N. fulvus* which has the nucleolar organiser region (NOR) terminal on pair five (Mahony and Roberts 1986). The NOR is medial on the long arm of chromosome five in *N. albipes* (Figure 3).

Genetic differentiation

Details of allele frequencies at individual loci are available on request from the senior author. There were fixed differences at two loci (LDH3 and MDH), with only a single allele in each species. No heterozygotes were detected confirming the reproductive isolation of *N. albipes* and *N. pelobatoides*. Nei's (1978) genetic distance between the two species (all populations combined for each) was 0.168 — a level consistent with species level differentiation in other organisms (Thorpe 1982; Richardson *et al.* 1986).

Distribution

Figure 4 shows the known distribution of *N. albipes*. The species occurs in the eastern and south-eastern wheat-belt, extending into adjacent western goldfields. There are no records west of a line running south from Quairading, and despite extensive field work in the Merredin area over the last six years, we have never observed this species north of

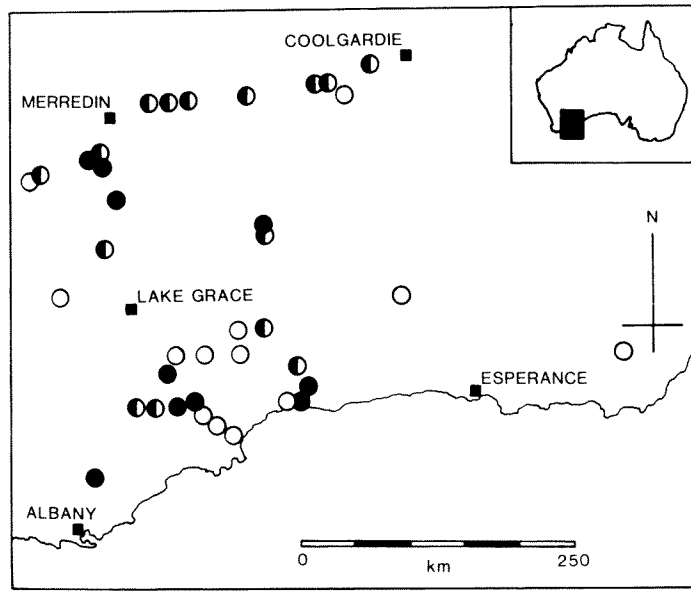

Figure 4. Distribution of *N. albipes:* closed circles, call recordings; half open circles, calls heard; open circles, specimen records.

Narembeen. *Neobatrachus albipes* is sympatric with *N. pelobatoides* and *N. kunapalari* throughout most of this range, but *N. kunapalari* does not occur in the area near the Stirling Range.

Comparisons with other species

Distinguished from all other species except *N. pelobatoides,* by the colour of the metatarsal tubercle: black in other species, unpigmented or edged with light brown in *N. pelobatoides* and *N. albipes.* Distinguished in life from *N. pelobatoides* by presence of white pigment in skin on upper surface of foot. Upper foot skin lacks pigment in *N. pelobatoides* making foot the colour of the underlying flesh in life (see Figure 5). This difference is not apparent in preserved material. Also distinguished by male call: *N. albipes* has a lower pulse number but higher pulse rate and dominant frequency than *N. pelobatoides.*

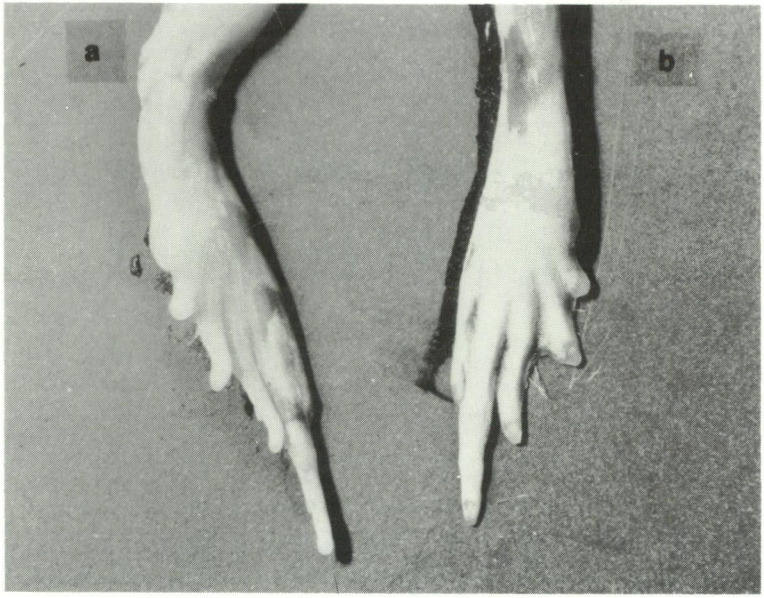

Figure 5. The upper surface of the foot in (a) *N. pelobatoides* (WAM R101178) and (b) *N. albipes* (WAM R101185) showing the difference in pigmentation. Photograph is of freshly killed material before preservation.

The combination of karyotype, electrophoretic and call structure data leave us in no doubt of the specific distinction of *N. albipes* from the broadly sympatric *N. pelobatoides* and *N. kunapalari.* Morphologically, however, these species are not markedly different and caution should be exercised in the allocation of preserved material. The following comparative notes may help with the correct identification of preserved specimens.

General morphology

Colour of metatarsal tubercle generally distinguishes *N. albipes* (13 of 16 unpigmented, three edged with brown), N. pelobatoides (13 of 15 edged with brown, two unpigmented), and *N. kunapalari* (10 black, four edged with black or dark brown). Mid-dorsal stripe generally present in *N. pelobatoides* (11 complete, two partial, two absent), generally absent in *N. albipes* (one present, one partial, 14 absent) but variable in *N. kunapalari* (one present, one partial, 14 absent). Light "V", or a derivative of this marking, between eyes always clear in *N. albipes* but never so obvious in *N. pelobatoides* and not present in *N. kunapalari.* Darker markings on the dorsum usually larger with clearly defined edges in *N. pelobatoides:* edges of markings diffuse in *N. albipes.* Darker markings on dorsum of *N. kunapalari* smaller than in *N. albipes* and also have a diffuse edge. In frogs where the black pigmentation of the nuptial pads was obvious on the first two fingers, we scored the presence of a nuptial pad on the third finger: *N. albipes,* two present, 11 absent; *N. pelobatoides,* 10 present, one absent; and *N. kunapalari,* seven present and five absent.

Morphometrics

Anova on all measured variables indicate significant differences in several variables, with *N. albipes* and *N. pelobatoides* similar but smaller than *N. kunapalari* (SV, HL, EL, T, F, EN, IN: Table 2, Fisher's multiple comparison test). For the three variables showing significant differences between all three species (HW, AIO, PIO; Table 2), *N. albipes* was intermediate between *N. kunapalari* and *N. pelobatoides. N. pelobatoides* has a longer snout but smaller tympanum than both *N. kunapalari* and *N. albipes* (NS, TW, TH; Table 2).

Table 2. One way analyses of variance for 13 metric variables amongst three species: *N. albipes, N. kunapalari* and *N. pelobatoides.* Superscripts (^{1}, ^{2}, ^{3}) indicate means that do not differ significantly using Fisher's multiple comparison test. All analyses have 2,42 degrees of freedom. Asterisks indicate probabilities: *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$,

Variable	F value	*N. kuna.*	*N. pelo.*	*N. albipes*
S-V	44.9***	52.0^1	39.6^2	39.4^2
HW	100.9***	21.9^1	15.6^2	16.9^3
HL	81.5***	17.7^1	13.6^2	14.0^2
EL	27.3***	7.9^1	6.1^2	6.4^2
ant. I-O	30.1***	8.4^1	6.2^2	7.1^3
post. I-O	57.9***	15.9^1	11.5^2	13.2^3
E-N	38.9***	3.7^1	2.7^2	2.6^2
I-N	20.7***	3.1^1	2.5^2	2.6^2
N-S	3.3*	1.6^1	1.9^2	1.6^1
tymp. H	7.6**	4.4^1	3.6^2	4.0^1
tymp. W	13.5***	3.6^1	2.8^2	3.4^1
T	9.1***	16.9^1	14.8^2	14.1^2
F	36.2***	32.0^1	25.3^2	24.9^2

In summary, *N. albipes* has a shorter snout, larger tympanum and broader head than *N. pelobatoides* of comparable size. *N. kunapalari* is generally larger than both other species.

Discussion

The discovery of another species of *Neobatrachus* from south-western Australia takes the number of species in this genus to 10: four tetraploid forms, *N. sudelli, N. centralis, N. kunapalari* and *N. aquilonius;* and six diploid forms, *N. pelobatoides, N. pictus, N. sutor, N. wilsmorei, N. fulvus* and *N. albipes.* Although each of these forms is readily distinguished from sympatric congeners using a combination of karyotype and call only *N. wilsmorei* is easily distinguished by external morphology. This, coupled with compex patterns of sympatry and parapatry has often made identification difficult. The holistic approach to species recognition adopted here, combining data from call, karyotype and allozyme electrophoresis allows unambiguous delineation of species boundaries. Importantly it also facilitates the recognition of consistent, albeit slight, morphological differences between species, thereby allowing easier identification of field and museum specimens.

Acknowledgements

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Appendix 1. Presumptive gene loci surveyed for electrophoretic variation in *N. albipes* and *N. pelobatoides.* Enzymes are numbered in order of their relative cathodal migration. Buffers are described in Richardson, Baverstock and Adams (1986).

Presumptive gene locus	Buffer	Tissue
Enzymic loci		
Acid phosphatase	TEB	liver
Creatine kinase	TEB	muscle
Esterase	TEB	muscle
Glutamate dehyrodgenase	TEB	muscle
Glutamate-oxaloacetate transaminase	TEB	muscle
Isocitrate dehydrogenase 1, 2	TM	liver
Lactate dehydrogenase 1, 2, 3	TM	liver
Leucyl amino peptidase	TC8	liver
Leucyl glysine peptidase	TEB	muscle
Leucyl tyrosine peptidase	TEB	muscle
Malic enzyme	TEB	muscle
Malate dehydrogenase	TM	muscle
Mannose-6-phosphate isomerase	TEB	muscle
6-phosphoglucose dehydrogenase	TM	liver
Phosphoglucomutase	TEB	muscle
Phosphoglucose isomerase	TC6	muscle
Super oxide dismutase 1, 2	TM	muscle
Non-enzymic loci		
General protein 1-4	TEB	muscle

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