

Notes on hemipenial morphology and its phylogenetic implications in the Pygopodidae Boulenger, 1884

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Abstract. The Pygopodidae is a near-endemic Australian family of limb-reduced, imbricately scaled gekkotans, currently comprising 44 species (43 in Australia, 2 in New Guinea). Phylogenetic studies of the Pygopodidae have enlisted molecular, morphometric, and osteological methods to resolve intergeneric and interspecific relationships, however, only one publication details the morphology of pygopodid hemipenes, providing data on four species from three genera. Here, we augment these initial observations, by describing hemipenial condition in an additional 19 species across four genera, bringing current totals to 23 pygopodid species across five of seven recognized genera. Focused sampling of the genus *Delma* allows us to identify species groups based on hemipenial shape and ornamentation, and general morphology of cloacal spurs. We identify the presence of a single-lobed hemipenis in both *Aprasia* and *Delma*, and hypothesize this modification in reproductive anatomy has occurred twice independently in pygopodid evolution.

Key words. Gekkota, Australia, hemipenes, systematics, cloacal spur, morphology.

INTRODUCTION

Diversity within a Divergent Lineage

Near-limbless geckos of the Family Pygopodidae represent a unique radiation in Australian biogeographic history. The snake-like pygopodids are characterized by an absence of forelimbs, imbricate body scales, and reduction of hindlimbs (Cogger 2014; Wilson & Swan 2013). Current taxonomy recognizes 44 species across seven genera; *Aprasia* Gray 1839 (14 spp.), *Delma* Gray 1831 (21 spp.), *Lialis* Gray 1835 (2 spp.), *Ophidiocephalus* Lucas & Frost 1897 (1 sp.), *Paradelma* Kinghorn 1926 (1 sp.), *Pletholax* Cope 1864 (1 sp.), and *Pygopus* Merrem 1820 (6 spp.). Although limb reduction is not a novel adaptation in squamate evolutionary history (Anniellidae, Anguillidae, Cordylidae, Dibamidae, Gymnophthalmidae, amphisbaenids and snakes), pygopodids are perhaps the most ecologically diverse limbless squamates exclusive of snakes (Gamble et al. 2015; Wiens et al. 2006). Current understanding of the ecology and phylogenetics (Brennan et al. 2016; Jennings et al. 2003; Oliver & Sanders 2009) suggests the Pygopodidae comprises: two independent burrowing lineages – *Aprasia* and *Ophidiocephalus*; shrub-swimmers – *Delma concinna*, *Pletholax*; squamate-specialist ambush predators – *Lialis*; a morphologically conservative genus of arthropod generalists – *Delma*; arachnid-specialists – *Pygopus*; and an insectivore with nectivorous habits – *Paradelma*.

Despite morphometric, molecular, and osteological assessments of the Pygopodidae, intergeneric and many interspecific relationships remain poorly understood (Hutchinson 1997; Jennings et al. 2003; Kluge 1974; Kluge 1976; Maryan et al. 2007; Oliver et al. 2010; Shea 1987; Shea 1991; Underwood 1957), and no phylogenetic study has included hemipenial characters. Böhme's (1988) description of hemipenes across squamate lineages remains an invaluable contribution to the understanding of the hemipenial condition, but with coverage of just four pygopodid species across three genera, it remains too incomplete for any phylogenetic use. Here, we present a more inclusive look at the hemipenial morphology of the Family Pygopodidae as a whole, with heavy sampling for the genus *Delma*, and aim to elucidate the synapomorphies shared by monophyletic groups within the family. The addition of Böhme's descriptions to our dataset proved extremely valuable, contributing observations on species which were inaccessible for this study.

Monophyly of the Pygopodidae and Previous Phylogenetic Study

Externally, pygopodids differ substantially from the plesiomorphic gekkotan body plan, however, evidence for the close relationship between these groups has been recognized and supported for a considerable period of time (Boulenger 1885; Greer 1989; McDowell & Bogert 1954;

Miller 1966; Shute & Bellairs 1953; Underwood 1957; Wever 1974). Recent comprehensive morphological assessment (Gauthier et al. 2012) correctly affiliated the Pygopodidae with the Gekkota, the only limbless squamate lineage to be accurately phylogenetically placed. Molecular studies have also solidified the position of pygopodids within the Gekkota (Feng et al. 2007), as well as within the Pygopodoidea (Gamble et al. 2012; Oliver & Sanders 2009), validating Böhme's (1988) preliminary hemipenial assessment of the Pygopodidae. While recent morphological (Daza & Bauer 2012) and molecular studies have elucidated the sister relationship between pygopodids and carphodactylids, intergeneric relationships within the Pygopodidae have varied greatly, with no single topology receiving overwhelming support (Daza & Bauer 2012; Jackman et al. 2008; Jennings et al. 2003; Oliver & Bauer 2011; Oliver & Sanders 2009). Morphological methods for phylogenetic analysis have also failed to create a single, favoured tree, and have conflicted strongly with molecular trees. As a result of the great phenotypic diversification between pygopodid genera, and conservatism within genera, previously assessed morphological characters are largely uninformative at the intergeneric level, and highly susceptible to homoplasy at the interspecific level (Kluge 1974; Kluge 1976; Kluge 1987). Additionally, current estimates of interspecific (Jennings et al. 2003) and intergeneric (Gamble et al. 2015) relationships within the Pygopodidae are largely influenced by potentially misleading mitochondrial data (Brennan et al. 2016). Molecular phylogenetic views within this paper follow the results of Brennan (Brennan et al. 2016), whilst taking into account the systematic results of previous pygopodid, pygopodoidean, and gekkotan research.

Hemipenial Morphology and Systematics

Morphological characters of male squamate intromittent organs provide sufficient systematic characteristics for inferring phylogenetic relationships (Arnold 1986a; Böhme 1988; Branch 1982; Branch 1986; Köhler et al. 2012). Historically, numerous studies have discussed and relied upon the phylogenetic signal of hemipenial morphology, beginning with Cope (1896) (Arnold 1986a; Arnold 1986b; Böhme 1971; Branch 1982; Branch 1986; Cope 1895; Dowling 1967; Dowling & Savage 1960; Keogh 1999; Klaver & Böhme 1986; Köhler et al. 2012; McCann 1946). Distinct morphological characters and ornamentation, presumed low intraspecific variation, and the rapid evolutionary trend of male genital morphology in relation to other morphological characters, make the study of hemipenial morphology a particularly valuable tool for systematists (Böhme 1988; Eberhard 1985; Keogh 1999; Köhler et al. 2012). Copulatory organs are diverse in their morphology, with various characters which can be described, count-

ed, and scored, including size, shape, and ornamentation (Dowling & Savage 1960; Keogh 1999). Despite lack of resolution at deeper taxonomic levels, comparative phylogenetic study of the hemipenes is a great tool for specific and generic levels due to extremely low intraspecific variation. Ontogenetic and seasonal variation tied to reproductive activity has been recorded from some lacertids, iguanids, and chameleons (Böhme 1988), and more recently in the gecko genus *Uroplatus* (Glaw et al. 2006). However, ontogenetic change generally influences size, and not shape or ornamentation. While various other morphological characters or systems may be artificially influenced by homoplasy via factors of natural history; ecology, diet, or locomotion, hemipenial morphology appears distanced from these pitfalls (Arnold 1986b; Böhme 1971; Böhme 1988; Branch 1986; Dowling 1967; Keogh 1999; Klaver & Böhme 1986).

Admittedly, intraspecific differences in hemipenial morphology have been identified among several snake taxa: *Calamaria lumbricoidea* (Inger & Marx 1962), *Candoia* (McDowell 1979), *Oxybelis aeneus* (Keiser 1974), and *Siphlophis* (Zaher & Prudente 1999). Perhaps due to a dearth of studies of this kind, no published research has included molecular phylogenetics to support instances of intraspecific hemipenial variation. In the first three of these, variation is strongly associated with geographic distribution, and as such, may be the result of independently evolving lineages. In fact, instances of proposed intraspecific hemipenial variation within *Iphis* (Nunes et al. 2012) actually highlight the value of molecular phylogenetics to address cryptic speciation and accurately identify species diversity.

Hemipenial diagnosis within the Gekkota began with Cope (1896), in the description of hemipenes from five currently recognized genera – *Cyrtodactylus*, *Coleonyx*, *Phyllodactylus*, *Tarentola*, and *Thecadactylus*. Since then, several studies have included observations and assessments of gekkotan hemipenes with phylogenetic implications. Standardly, gekkotan intromittent organs are bilobed, and often asymmetrical, with varying arrays of ornamentation. The New World sphaerodactylid genus *Aristelliger*, possesses a baculum-like structure, which is unique among gekkotans, described and illustrated first by Kluge (1982), as a spiny ossification with a serrated edge, which extends distally from the apex of each lobe (Rösler & Böhme 2006). Exclusive of Böhme (1988), however, comparative descriptions of the genital morphology of pygopodids are lacking. Additionally, cloacal spurs, and post-cloacal bones and sacs are often associated with reproductive morphology, and their presence in gekkotans was reviewed extensively by Kluge (1982), and has been sporadically commented upon (Bastinck 1986; Russell 1977; Russell & Rosenberg 1981).

In his description of *Aprasia*, *Delma*, and *Pygopus* genera, Böhme interprets the position and state of calyces

of *Pygopus* as a plesiomorphic character, supporting Kluge's (1974, 1976) placement of this genus as the basal-most member of the family. Using Kluge's (1976) subfamilial groups, the Pygopodinae (*Delma* and *Pygopus*) and Lialisinae (*Aprasia*, *Lialis*, *Ophidiocephalus*, *Pletholax*), Böhme identifies conflict within this ranking by way of similarity in ornamentation – or lack thereof – in *Aprasia* and *Delma*. He suggests the nude nature of *Aprasia* and *Delma* hemipenes may reflect a close evolutionary history, or instead be the result of an ornamental-reversal, or hemipenial simplification, as also seen in *Brookesia* (Klaver & Böhme 1986). Current understanding of intergeneric relationships based on molecular results however refutes a sister taxa relationship between *Aprasia* and *Delma*, and phylogenetic conclusions regarding intergeneric relationships within the Pygopodidae by Böhme (1988) should be viewed as an artifact of insufficient sampling. Despite deep external morphological divergence from a tetrapodal ancestor, Böhme does identify pygopodid hemipenes as distinctly gekkotan. In order to continue in the same vein as Böhme's work, here we diagnose and describe the hemipenial morphology of a number of Australian pygopodids, particularly of the genus *Delma*, adding to current documentation of pygopodid hemipenes. Images, descriptions, and preparation of these organs may aid in future phylogenetic assessments of the Pygopodidae and future work in this area will provide additional discernible characteristics for recognizing, delimiting, and describing new species.

MATERIALS & METHODS

Hemipenial Preparation

Intromittent reproductive organs have independently arisen in a number of vertebrate groups – *Ascaphus* frogs, crocodilians, chelonians, mammals, chondrichthyes – however paired inverted hemipenes represent a synapomorphy of squamate reptiles (Greer 1989). Hemipenes themselves are paired tubular organs, which when not in use are retracted and stored within the body in “inside-out” fashion (Dowling & Savage 1960). When tucked inside the body, they are inverted like a glove, and upon stimulation, are engorged and pushed outside of the body – the inside in the stored state becoming the outer wall upon eversion. Upon being everted, hemipenes may protrude laterally, anteriorly, or posteriorly from the cloaca, and may be decorated with a number of ornaments including calyces (calyculi), flounces, spikes, hooks, and lobes. We generally follow terminology as proposed by Dowling & Savage (1960), however refer to Keogh (1999) for moderate adjustments. Accurate morphological hemipenial assessment – particularly of apical lobes – requires a fully everted hemipenis, either preserved *in situ*, or manually

everted as described by Pesantes (1994). We acquired specimens on loan from the Western Australian Museum (Table 1) with completely everted organs, and only in the absence of such specimens, did we select individuals with incompletely everted hemipenes. We abstained from destructive practices by excluding species for which entirely or partially everted organs could not be obtained. Incompletely everted hemipenes were injected to capacity with 1% KOH solution and left for one hour to soften tissue for manual eversion. After an hour, KOH was removed from organs, and liquid 1.5% agarose gel dyed with alizarin red was injected via 30 gauge hypodermic syringe, to complete volume. Dyed agarose gel set inside the hemipenes instantly, and red coloration allowed for increased contrast of structures, aiding observation and imaging.

Morphology of pygopodid hemipenes is severely reduced in complexity as well as physical size, when compared to other squamate taxa (Arnold 1986a; Branch 1986; Dowling 1967; Hoskin 2011; Köhler et al. 2012). Calcareous spines, spicules, calyces, and flounces present in other squamates appear absent in sampled pygopodids, making staining structures difficult, and imaging of pygopodid hemipenes a challenge. As a result, we have reduced the number of characters addressed by Keogh (1998), and provide brief overall descriptions of organs of each species.

Characters and Character States

Shape: Single (S), bi-lobed symmetrical (BS), bi-lobed asymmetrical (BA), or bi-lobed with additional median lobe (T).

Ornamentation: Nude (N), undifferentiated (UD) ornamentation is homogeneous and uniform over the entire surface of the hemipenis (e.g., spines only). Differentiated (D) ornamentation is defined as two or more differing types of ornaments (spines and micro-ornamentation).

Base: Ornamented (O) or nude (N).

Terminal sulcus: Sulcus spermaticus terminates at apex of lobe (TSA), at lateral edge of lobe (TSL), or medially as a sulcal pad (TSP). In asymmetrical conditions, the smaller lobe is listed first/preceded by the larger lobe.

Spurs: General shape of the spur is rounded (R), pointed (P), flattened (F) or terminates in comb-like projections (C). Direction in which the spur projects is either posteriorly (P), dorsally (D) or posteriodorsally (U). A rounded spur which points dorsally would be marked RD.

Lack of intraspecific variation in hemipenial morphology across species sampled suggests the number of individuals examined should provide sufficient for our interest (Arnold 1986a; Böhme 1988; Keogh 1999). The relatively limited breadth of this study (22 spp., 5 genera)

Table 1. Taxa, locality data, and sources of specimens examined in this study. States refers to Australian states.

*UMMZ = University of Michigan Museum of Zoology; WAM = Western Australian Museum; ZFMK = Zoologisches Forschungsmuseum A. Koenig. Specimens for which coordinates are given as NA are those examined in the earlier study of Böhme (1988).

Taxon	Collection*	State	Locality	Latitude	Longitude
<i>Aprasia haroldi</i>	WAM R163615	WA	Dirk Hartog Island	25°41'60"S	113°0'0"E
<i>Aprasia parapulchella</i>	WAM R144181	WA	4 km S Collie	33°22'03"S	116°13'49"E
<i>Aprasia parapulchella</i>	WAM R153920	WA	Bindoon Military Training Area	31°20'31"S	116°15'39"E
<i>Aprasia pulchella</i>	UMMZ 131241	WA	Canning Dam	NA	NA
<i>Aprasia repens</i>	WAM R144255	WA	Burns Beach	31°43'04"S	115°45'59"E
<i>Aprasia repens</i>	WAM R153978	WA	Bindoon Military Training Area	31°11'42"S	116°18'26"E
<i>Aprasia smithi</i>	WAM R120652	WA	8 km NW Mardathuna Homestead	24°25'44"S	114°30'00"E
<i>Delma australis</i>	WAM R112667	WA	Ponier Rock	32°56'00"S	123°30'00"E
<i>Delma australis</i>	WAM R116276	WA	Kalbarri	27°51'00"S	114°10'00"E
<i>Delma australis</i>	WAM R135108	WA	Bullabulling	30°51'59"S	120°54'24"E
<i>Delma australis</i>	WAM R140395	WA	90 km NE Wubin	29°31'23"S	117°10'10"E
<i>Delma borea</i>	WAM R154148	WA	Barrow Island	20°47'18"S	115°27'43"E
<i>Delma borea</i>	WAM R158010	WA	Koolan Island	16°08'45"S	123°44'57"E
<i>Delma butleri</i>	WAM R120322	WA	7 km E Cape Cuvier	24°13'26"S	113°27'41"E
<i>Delma butleri</i>	WAM R120819	WA	25 km SSE Peron Homestead	26°03'00"S	113°37'00"E
<i>Delma butleri</i>	WAM R123911	WA	Bulong	30°45'00"S	121°48'00"E
<i>Delma desmosa</i>	WAM R134414	WA	Lake MacKay	22°26'47"S	128°17'33"E
<i>Delma elegans</i>	WAM R135462	WA	Mount Brockman	22°28'00"S	117°18'00"E
<i>Delma fraseri</i>	WAM R115138	WA	Ken Hearst Park	32°04'60"S	115°52'60"E
<i>Delma fraseri</i>	WAM R135503	WA	Redcliffe, Perth Suburb	31°56'00"S	115°52'00"E
<i>Delma fraseri</i>	WAM R141191	WA	15 Km NNE Lancelin	30°59'31"S	115°23'43"E
<i>Delma fraseri</i>	WAM R154039	WA	Muchea Air Weapons Range	31°38'16"S	115°55'31"E
<i>Delma grayii</i>	WAM R154364	WA	Hindmarsh Nature Reserve	31°17'00"S	117°02'00"E
<i>Delma grayii</i>	WAM R156220	WA	Ballajura	31°51'11"S	115°55'11"E
<i>Delma haroldi</i>	WAM R138951	WA	West Angelas	23°11'42"S	118°36'54"E
<i>Delma haroldi</i>	WAM R154831	WA	Goldsworthy, Shay Gap Road	20°25'45"S	120°11'11"E
<i>Delma hebesa</i>	WAM R144236	WA	Bandalup Hill	33°40'29"S	120°23'54"E
<i>Delma hebesa</i>	WAM R172285	WA	Scaddan	33°26'27"S	121°43'17"E
<i>Delma inornata</i>	UMMZ 131156	VIC	Numurkah	NA	NA
<i>Delma inornata</i>	UMMZ 131186	NSW	Finley	NA	NA
<i>Delma nasuta</i>	WAM R154288	WA	Fortescue Marsh	21°48'09"S	118°54'39"E
<i>Delma nasuta</i>	WAM R157568	WA	Robe River	21°40'26"S	115°53'21"E
<i>Delma pax</i>	WAM R135337	WA	Cape Lambert	20°48'36"S	116°56'31"E
<i>Delma pax</i>	WAM R166212	WA	Mount Whaleback	23°19'41"S	120°01'07"E
<i>Delma petersoni</i>	WAM R165873	WA	Queen Victoria Spring	29°19'11"S	124°31'28"E
<i>Delma petersoni</i>	WAM R165874	WA	Queen Victoria Spring	29°19'11"S	124°31'28"E
<i>Delma tealei</i>	WAM R153811	WA	Cape Range National Park	22°03'49"S	114°00'42"E
<i>Delma tincta</i>	WAM 135487	WA	Urala Station	21°46'58"S	114°52'11"E
<i>Delma tincta</i>	WAM 141584	WA	1 km N Quobba Homestead	24°22'24"S	113°24'19"E
<i>Delma tincta</i>	WAM 146589	WA	228 km SSW Port Hedland	22°20'24"S	119°00'00"E
<i>Lialis burtonis</i>	WAM 110652	WA	Shay Gap Road, Goldsworthy	21°43'00"S	122°14'00"E
<i>Lialis burtonis</i>	WAM 154003	WA	Muchea Air Weapons Range	31°38'32"S	115°55'03"E
<i>Lialis burtonis</i>	WAM 154007	WA	Muchea Air Weapons Range	31°38'32"S	115°55'03"E
<i>Pletholax g. gracilis</i>	WAM 106172	WA	Marangaroo	31°48'00"S	115°50'00"E
<i>Pletholax g. gracilis</i>	WAM 137463	WA	Cervantes	30°45'03"S	115°12'11"E
<i>Pygopus lepidopodus</i>	ZFMK 21290	NSW	Sydney	NA	NA
<i>Pygopus nigriceps</i>	UMMZ 131174	SA	Innaminka	NA	NA

Table 2. Summary of hemipenial characters of pygopodid geckos examined. *denote taxa examined by Böhme (1988). Symbols as follows: S=Single-lobed, BA=Bi-lobed asymmetrical, BS=Bi-lobed symmetrical, UD=Undifferentiated ornamentation, D=Differentiated ornamentation, N=Nude of ornamentation, TSA=Sulcus terminates at apex of lobe, TSL=Sulcus terminates at lateral edge of lobe, TSP=Sulcus terminates medially into broad sulcal pad. See materials and methods for descriptions of characters, and Table 1 for material examined.

Taxon	No.	Shape	Ornamentation	Base	Terminal Sulcus	Length (mm)	SVL (mm)	Spurs
<i>Aprasia haroldi</i>	1	S	UD	N	TSA	2.58	106	—
<i>Aprasia parapulchella</i>	1	BA	UD	N	TSA/TSP	1.82–2.08	140	—
* <i>Aprasia pulchella</i>	1	BA	UD	—	TSA	—	120	—
<i>Aprasia repens</i>	2	S	UD	O	TSA/TSP	3.41–4.28	126	—
<i>Aprasia smithi</i>	1	S	D	N	TSA/TSP	3.67	128	—
<i>Delma australis</i>	4	S	UD	N	TSP	4.33–5.73	88	FU
<i>Delma borea</i>	2	BA	UD	N	TSP/TSA	4.38–5.95	95	RP
<i>Delma butleri</i>	3	BS	UD	N	TSL/TSP	5.48–8.59	96	CP
<i>Delma desmosa</i>	1	BA	UD	N	TSL/TSA	4.96	90	RP
<i>Delma elegans</i>	1	BA	UD	N	TSL/TSP	5.5	97	RP
<i>Delma fraseri</i>	2	BA	N	N	TSL/TSP	5.57–6.57	128	PU
<i>Delma grayii</i>	2	BS	UD	N	TSL/TSP	5.62–7.86	121	PP
<i>Delma haroldi</i>	1	BA	UD	N	TSL/TSP	3.83–4.22	75	CP
<i>Delma hebesa</i>	2	S	UD	N	TSP	3.58–3.9	85	FU
* <i>Delma inornata</i>	3	BA	UD	N	—	—	133	—
<i>Delma nasuta</i>	2	BA	UD	N	TSL/TSA	4.28–5.38	112	CP
<i>Delma pax</i>	2	BA	UD	N	TSL/TSA	5.55–5.96	98	RP
<i>Delma petersoni</i>	2	BS	N	N	TSL/TSP	6.06–7.68	128	PU
<i>Delma tincta</i>	3	BA	UD	N	TSL/TSA	3.36–4.19	92	RP
<i>Lialis burtonis</i>	4	B—	D-micro and calyces	N	—	—	290	—
<i>Pletholax gracilis</i>	2	BA	D-micro and spines	N	TSA	4.07–4.88	90	—
* <i>Pygopus lepidopodus</i>	1	T	D-micro and calyces	O	TSA	—	274	—
* <i>Pygopus nigriceps</i>	1	T	D-micro and calyces	O	TSA	—	—	—

allows us to describe each species independently. Closely related species, or those similar in hemipenial morphology may cross reference one another. Several species (*Aprasia parapulchella*, *Delma pax*, *Lialis burtonis*), are included despite incompletely everted hemipenes. These examples were not everted when fixed, and could not be manually everted, however still provide some phylogenetic utility. Characters which could not be scored for these taxa (apical characters and ornamentation) are symbolized by “—” in Table 2.

RESULTS

Hemipenial Descriptions

*Denotes taxa examined and described by Böhme (1988)

Aprasia haroldi Storr, 1978

Fully everted; extremely minute, single lobe covered entirely in micro-ornamental stippling except for the sulcus.

Base nude until constriction at bottom of lobe, sulcus broad and shallow. Hemipenis boxing-glove shaped, highly asymmetrical, proximal lobe miniaturized in relation to distal lobe.

Aprasia parapulchella Kluge, 1974

Incompletely everted; bi-lobed. Sulcus narrow at base, until constriction at base of fork, sulcus becomes broad and shallow along lengths of lobes. Light micro-ornamentation (stippling) of asulcal side, with sulcus nude.

Aprasia pulchella Gray, 1839*

Fully everted; bi-lobed. Asymmetrical, inner lobe (as prepared in-situ) larger. Sulcus spermaticus forked, with much longer branch on inner lobe. Little ornamentation can be observed beside the presence of a pustular epithelium, and absence of calyx bearing surfaces.

Aprasia repens (Fry, 1914) (Figs 1–1a, b)

Fully everted; single lobe. Asulcal and lateral faces com-



Fig. 1. Asulcal (a) and sulcal (b) views hemipenes of eight species of pygopodids: 1) *Aprasia repens* WAMR144255; 2) *Delma australis* WAMR112667; 3) *Delma elegans* WAMR135462; 4) *Delma haroldi*; 5) *Delma nasuta* WAMR154288; 6) *Delma petersoni* WAMR165873; 7) *Delma pax* WAMR135337; 8) *Pletholax gracilis* WAMR106172.

pletely covered with micro-ornamental stippling, including base. Slipper-shaped hemipenis, recurving posteriorly, similar in jai alai basket, as *A. smithi*. Sulcus narrow and deep until reaching lobe, becomes deep and wide, transitioning into sulcal pad, pad clearly surrounded by deep sulcal lips. Distinct nub at posterior facing edge of asulcal face, potential remnant of secondary lobe.

Aprasia smithi Storr, 1970

Fully everted; single lobe. Hemipenis is long, thin, and curves dorsally and against body wall after exiting cloaca, perhaps an artifact of the eversion process. Stippled with micro-ornamentation laterally, with stronger spines along asulcal ridge. The sulcus is wide and shallow, and

reaches apex. Proportions and shape cause the hemipenis to resemble a jai alai basket.

Delma australis Kluge, 1974 (Figs 1–2a, b)

Fully everted; single lobe. Boxing glove shape, extends laterally from cloaca and curls back towards midline. Both sulcate and asulcate surfaces covered in fine micro-ornamentation, with the exclusion of broad, shallow sulcus which does not reach apex.

Delma borea Kluge, 1974

Fully everted; bi-lobed. Strongly asymmetrical, posterior/ventral lobe elongate with truncate, nude apex, which is reached by sulcus. Dorsal/anterior lobe hammer

shaped, with sulcus reaching lateral face before opening onto sulcal pad. Base and lower quarter of lobes nude, but finely micro-ornamented across asulcal face. Terminus of longer lobe ends in flat disc, with sulcal lips strongly folding over, nearly closing over sulcus.

Delma butleri Storr, 1987

Presentation of *D. butleri* includes individuals from two geographically isolated populations suggested to represent cryptic species. Variation in hemipenial morphology between these two groups warrants further molecular study.

WAM 120322, WAM 120819 – Fully everted; bi-lobed. Lobes appear approximately equal in size, shallowly forked compared to other delmas, distinctly Y-shaped in comparison to T shape of *D. butleri* WAM 123911. Base nude until just prior to cleft, with micro-ornamentation covering asulcal face, becoming stronger laterally. Sulcus, buffered by deep sulcal lips, extends laterally beyond split of lobes, opening onto sulcal pad, facing dorsally.

WAM 123911 – Fully everted; bi-lobed. T split between lobes, with apex of lobes terminating more in points than the lobular end of above *D. butleri* samples. Sulcus is deep and narrow, forks at cleft of lobes, and extends laterally. Medial lateral faces of lobes (closest to one another) nude. Sulcus terminates laterally, opening out onto nude sulcal pad.

Delma desmosa Maryan, Aplin & Adams, 2007

Fully everted; bi-lobed and highly asymmetrical, similar in general shape to *D. tincta*, *D. elegans*, *D. pax*, and *D. borea*. Anterior/dorsal lobe much shorter, with hammerhead shape. Posterior/ventral lobe elongate, with truncate, nude tip, and strongly folded sulcal lips. Both sulcal and asulcal faces are ornamented.

Delma elegans Kluge, 1974 (Figs 1–3a, b)

Fully everted; bi-lobed. Asulcal face strongly stippled and micro-ornamented, but restricted to the lobes. Base nude, and lateral sides of lobes stippled. Anterior lobe much smaller and broader, but unlike the hammerhead shape of *D. pax*. Sulcus very deep and narrow on both lobes, extending laterally away from midline and not directly towards apex, becoming shallow at most distal edge of sulcal pad; sulcal pad nude.

Delma fraseri Gray, 1831

Fully everted; bi-lobed. Smaller lobe approximately one-third size of larger, however similar in shape, broad and spatulate, clover leaf shaped. Entire hemipenis bare, no strong stippling as in others. Sulcus narrow and deep, until reaching cleft of lobes, then becomes extremely shallow channel which diverts laterally away from the midline, and opens up into a shallow pad-like surface on each lobe.

Delma grayii Smith, 1849

Fully everted; bi-lobed. Both lobes approximately same size. Asulcal side only lightly stippled, following around to lateral edges. Sulcus deep and narrow, deepest at cleft between lobes, and becomes extremely narrow and channel-like as bifurcates and diverts away from midline. Lobe-sulcus-channel opens up onto broad, flat, pad covering most of sulcal side of lobe.

Delma haroldi Storr, 1987 (Figs 1–4a, b)

Fully everted; bi-lobed. Slightly asymmetrical, with posterior lobe smaller, but of similar overall shape. Sulcus is deep and narrow, and terminates laterally on each lobe, after splitting at lobe-fork. Sulcus opens up onto nude sulcal pad. Asulcal face only lightly ornamented, extending onto base. Spur projects posteriorly, and distal tip covered in several projects, giving it a comb-like appearance.

Delma hebasa Maryan, Brennan, Adams & Aplin, 2015

Fully everted; single lobe. Apex appears more truncate and lobe more bulbous than *australis*. Strongly ornamented on both sulcal and asulcal faces, with the exclusion of the sulcus. Sulcus sharply edged by lips, terminating prior to apex. Apex projects posteriorly substantially, but does not reach point found in *D. australis*.

Delma inornata Kluge, 1974*

Fully everted; bi-lobed. Hemipenis is bulb-shaped, with poorly differentiated apical lobes. The medial (in-situ) lobe is larger than lateral lobe. Sulcus is forked, and surface epithelium is covered by strongly pustule features, calyces absent.

Delma nasuta Kluge, 1974 (Figs 1–5a, b)

Fully everted; bi-lobed. Strongly asymmetrical lobes. Medial lobe (generally smaller in delmas with asymmetrical hemipenes) strongly cleft at distal end of sulcus, opening onto laterally-facing sulcal pad. Smaller lobe balloon shaped, much narrower at base. Lateral lobe larger, and truncate at terminus, with sulcus reaching apex. Sulcal lips on larger lobe strongly overlap sulcus. Asulcal face only lightly ornamented, base nude. Spurs project dorsally and posteriorly, and terminate in comb-like tips, not as pronounced as *D. haroldi*.

Delma pax Kluge, 1974 (Figs 1–7a, b)

Fully everted; bi-lobed. Dorsal lobe shorter and broader, hammerhead in shape, truncate, with sulcus reaching apex. Ventral lobe elongate, narrow, end truncate, covered in micro-ornamentation. Asulcal surface micro-ornamented laterally, nude medially. Sulcus deep and narrow, both lobes nude until beyond fork, with finely stippled tips.

Delma petersoni Shea, 1991 (Figs 1–6a,b)

Fully everted; bi-lobed. Deep cleft between approximately similar sized lobes. Surface almost entirely nude, similar to *D. fraseri*. A deep, narrow sulcus bisects at lobe-cleft, becomes narrow channels diverting away from mid-line and opening out into broad flat surface on sulcal side.

Delma tinca De Vis, 1888

Fully everted; bi-lobed. Asymmetrical lobes, smaller of the two hammerhead shaped. Both lobes, including asulcal faces, but excluding sulcal pads, are covered in fine micro-ornamentation which is strongest on asulcal face of large lobe. Sulcus narrow and deep, continues laterally from lobe-fork, to lateral edge of sulcal pad of shorter lobe, and to apex of elongate lobe. Smaller, hammer-shaped lobe broad and flattened, creating large sulcal face large composed of sulcal pad.

Lialis burtonis Gray, 1835

Incompletely everted; distinctly bi-lobed. Deep sulcus, with nude base. Asulcal and sulcal faces, excluding sulcus, covered in fine ornamentation.

Pletholax gracilis (Cope, 1864) (Figs 1–8a, b)

Fully everted; bi-lobed. Mitten shaped, with dorsal lobe substantially reduced, ventral lobe larger and elongate. Asulcal surface partially nude, but densely covered in micro-spines and ornamentation laterally. Hemipenial base nude until constriction at base of lobe division. The sulcus is narrow, but deep, and widens towards apices, sulcus reaches apex of each lobe.

Pygopus lepidopodus (Lacépède, 1804)*

Incompletely everted; specimen preserved in 1864, long before assessment by Böhme. Median lobe can be made out, reminiscent of *P. nigriceps*. Calyces on base are small and end prior to apex of lateral lobes.

Pygopus nigriceps (Fischer, 1882)*

Fully everted; tri-lobed. Hemipenis generally short and broad. Sulcus spermaticus divided into two long forks along the length of larger, divided lobes. Sulcus is a deep depression. A small, pear-shaped, undivided lobe sits between larger lateral main lobes. Asulcal face covered in small calyces, base covered in transverse calyces with beaded edges. The outer lateral lobes are covered with small deep calyces with prickly edges. Asulcal face of lobes smooth.

DISCUSSION

Systematic Implications of the Hemipenes of Pygopodids
Relative to other squamate groups, and even other gekkotans, pygopodid hemipenes are markedly simplified

in their overall morphology (Böhme 1988). Morphological reduction in hemipenial characters may be the result of an ancestral miniaturization event at the base of the pygopodid tree, as seen in *Brookesia* chameleons (Klaver & Böhme 1986). While in contrast, similarly small squamates such as gymnophthalmids have not seen this simplification in hemipenial morphology, pygopodid hemipenial size (length from cloaca to apex) relative to body length (SVL) is substantially smaller than that of the gymnophthalmid *Iphisa elegans* (Nunes et al. 2012). Reduction in hemipenial characters as a result of miniaturization in fossorial limbless squamates has been observed in the morphologically and ecologically similar typhlopids (Khan 1999; Thomas & Hedges 2007). This morphological simplification is most pronounced in the three species of *Aprasia* (*A. haroldi*, *A. repens*, *A. smithi*) and two *Delma* species (*D. australis*, *D. hebesa*) which exhibit single-lobed hemipenes. These species are among the smallest members of their respective genera, and the disjunct nature of this characteristic in the pygopodid tree suggests two independent evolutionary events. Presence of bi-lobed hemipenes in all five examined pygopodid genera, as well as the exclusive condition in carphodactylid and diplodactylid geckos, supports the pygopodid, pygopodoidean, and gekkotan ancestral hemipenial conditions as bi-lobed.

Mitochondrial and allozyme history suggests a basal split within *Aprasia*. *Aprasia haroldi*, *A. pulchella*, *A. repens*, and *A. smithi*, all examined here, are members of a single clade, closely related to *A. rostrata* as well as the recently described *A. clairae* (Maryan et al. 2013b) and *A. litorea* (Maryan et al. 2013a), for which no hemipenial assessment has been made. *Aprasia parapulchella* represents the sole observed member of the alternative, moderately divergent group (Fig. 2). *Aprasia pulchella*, which is closely related to the single-lobed group, exhibits the bi-lobed hemipenial condition, similar to the asymmetrically bi-lobed hemipenes of *A. parapulchella*, despite their distant relatedness within *Aprasia*. Incomplete molecular and hemipenial sampling of this genus necessitates additional assessment of *Aprasia* species to determine the ancestral hemipenial condition. Further study will determine if single-lobed hemipenes occur as a synapomorphy of the *A. fusca* group (*A. smithi*, *A. litorea*, *A. haroldi*, *A. clairae*, *A. repens*, *A. rostrata*, *A. fusca*).

Perhaps more surprising than morphological reduction in the diminutive fossorial *Aprasia*, is the occurrence of single-lobed hemipenes in the *Delma australis* group. Here, hemipenial reduction is noted in *D. australis* and the newly described *D. hebesa*, however the closely related *D. torquata* has not yet been assessed (Fig. 3). Close relations between *D. australis* and *D. torquata* suggest the potential for a similar hemipenial condition. Assessment of hemipenial morphology of *D. concinna* and *D. torquata* would contribute to the understanding of this group. Ob-

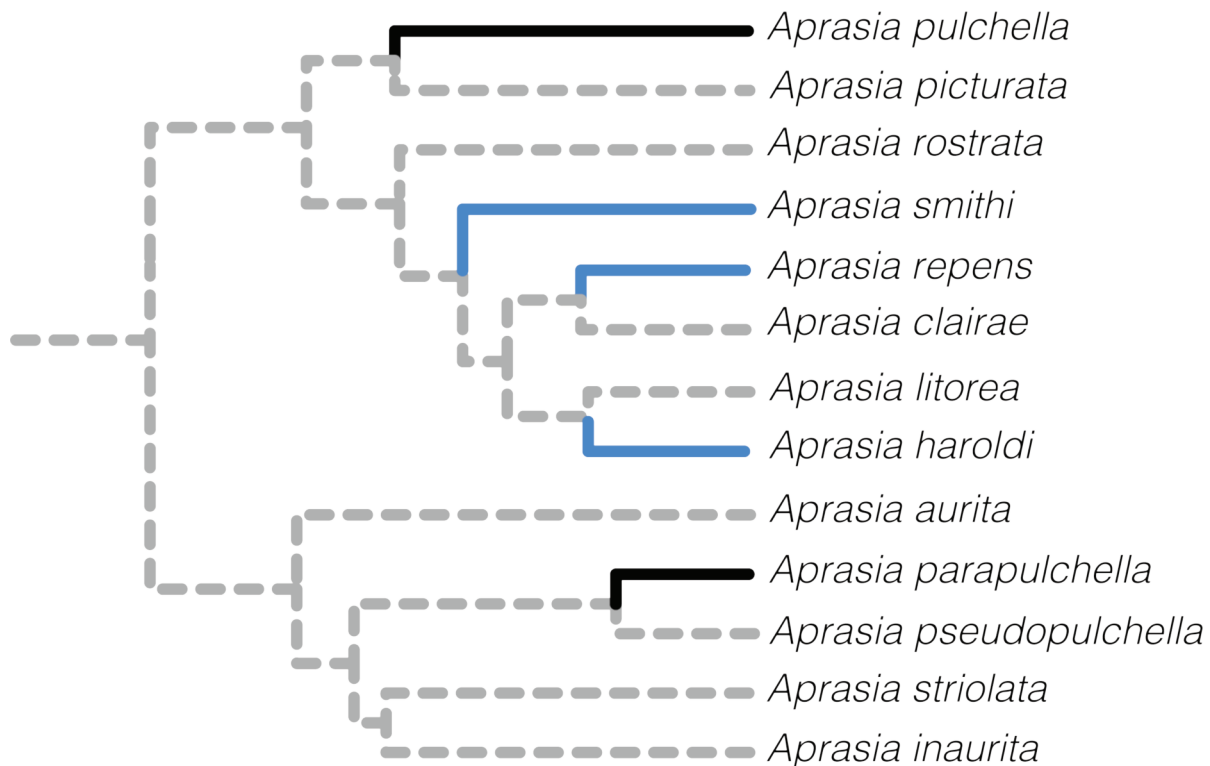


Fig. 2. Schematic phylogeny of *Aprasia* composed of mitochondrial data from Jennings et al. (2003) and allozyme data from Maryan et al. (2013a) and Maryan et al. (2013b). Grey dotted branches indicate lineages for which hemipenial morphology has not yet been assessed. Black branches indicate species which display bi-lobed hemipenes, and blue branches denote species with single-lobed hemipenes.

servation of single-lobed hemipenes in *D. concinna* or *D. torquata* would strongly support inclusion of these species in the *D. australis* clade, establishing a synapomorphy of this group.

Within the bi-lobed pygopodids, there remains considerable morphological distinction between genera, species groups, and individual species. Although members of *Aprasia* may exhibit either single- or bi-lobed hemipenes, regardless of overall shape, the sulcus spermaticus broadens out onto a sulcal pad, comprising most of the sulcal face of the hemipenis. The sulcal lips in *Aprasia* also strongly delineate the sulcus spermaticus, and the entirety of the asulcal and sulcal faces, including even the sulcal lips, are covered in fine micro-ornamentation. The sulcal pad terminates at the apex in both single- and bi-lobed species of *Aprasia*, and remains unornamented in all observed species.

The moderate to small sized, heavily nuchal banded *Delma* species of northern and northwestern portions of Australia *D. borea*, *D. desmosa*, *D. elegans*, *D. pax*, and *D. tincta*, can be distinguished by asymmetry not only in size, but in general shape of the bi-lobed hemipenes. In *D. borea*, *D. desmosa*, *D. pax*, and *D. tincta* a hammer-shaped

lobe extends anteriorly or proximally, while the second lobe, elongate, with a truncate apex and strongly folded sulcal lips, extends dorsally or distally. *Delma elegans* also exhibits a strongly asymmetrical design, however the disparity in shape between the anterior/medial and dorsal/distal lobes is less pronounced, and the dorsally projecting lobe is not elongate, nor is the apex truncate. Based on similarity in shape, asymmetry, and ornamentation, we suggest a close affinity among *D. borea*, *D. desmosa*, *D. pax*, and *D. tincta*, but are unable to further hypothesize systematics based on hemipenial morphology alone. *Delma elegans* appears to show a much simplified asymmetrical condition, potentially embodying the ancestral condition for this group.

Delma fraseri and *D. petersoni*, previously confused under a single species, display a nearly identical hemipenial condition, most likely the result of recent ancestry. Hemipenes of these two species are either weakly asymmetrical, or symmetrical in size and shape, both nude, with sulci that terminate laterally on sulcal pads. Based on mitochondrial data (Jennings et al. 2003), *D. grayii* has been recognized as sister taxon to *D. fraseri*, and despite similar hemipenial morphology – narrowly asymmetrical

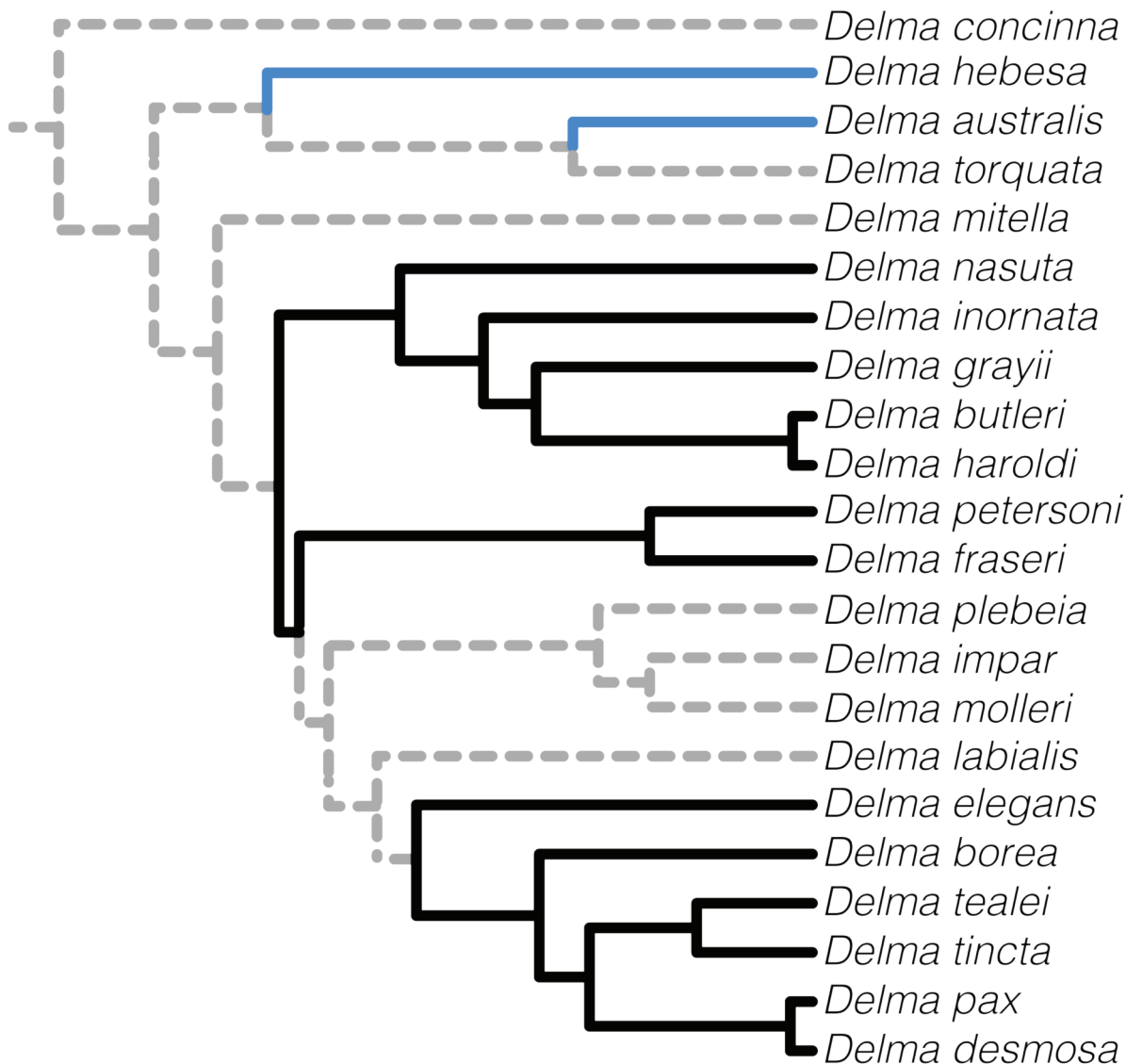


Fig. 3. Species tree phylogeny of *Delma* as inferred by nDNA data from Brennan et al. (2016). Grey dotted branches indicate lineages for which hemipenial morphology has not yet been assessed. Black branches indicate species which display bi-lobed hemipenes, and blue branches denote species with single-lobed hemipenes.

bulb-shaped lobes, sulcus terminating laterally in pads – nuclear DNA data (Brennan et al. 2016) suggests this sister relationship may instead be an artifact of a historical introgression event, the cause of such an event, or a bi-lobed, narrowly asymmetrical, bulb-shaped, largely unornamented hemipenis may represent the ancestral *Delma* hemipenial condition.

Moderate asulcal ornamentation, alongside a narrowly asymmetrical bi-lobed design unites and the *D. butleri* group (*D. butleri*, *D. grayii*, *D. haroldi*, *D. inornata*, *D. nasuta*). Except for *D. nasuta*, this group is typified by micro-ornamentation restricted to the lobes, distal to the

point of bifurcation of the sulcus. The sulcus terminates laterally onto shallow broad sulcal pads, which are also devoid of ornamentation. Within *D. butleri* we recognize two differing hemipenial conditions: large-bodied and lightly patterned *D. butleri* from the Carnarvon region (WAM120322, WAM 120819) display hemipenes similar to that of *D. haroldi*, whereas the more northern and inland *D. butleri* (WAM123911) shows a distinct shape and lack of ornamentation. Allopatry of distinct *D. butleri* populations and morphological differences highlight the potential of cryptic species, or possible intraspecific variation with this broadly distributed variable species. In com-

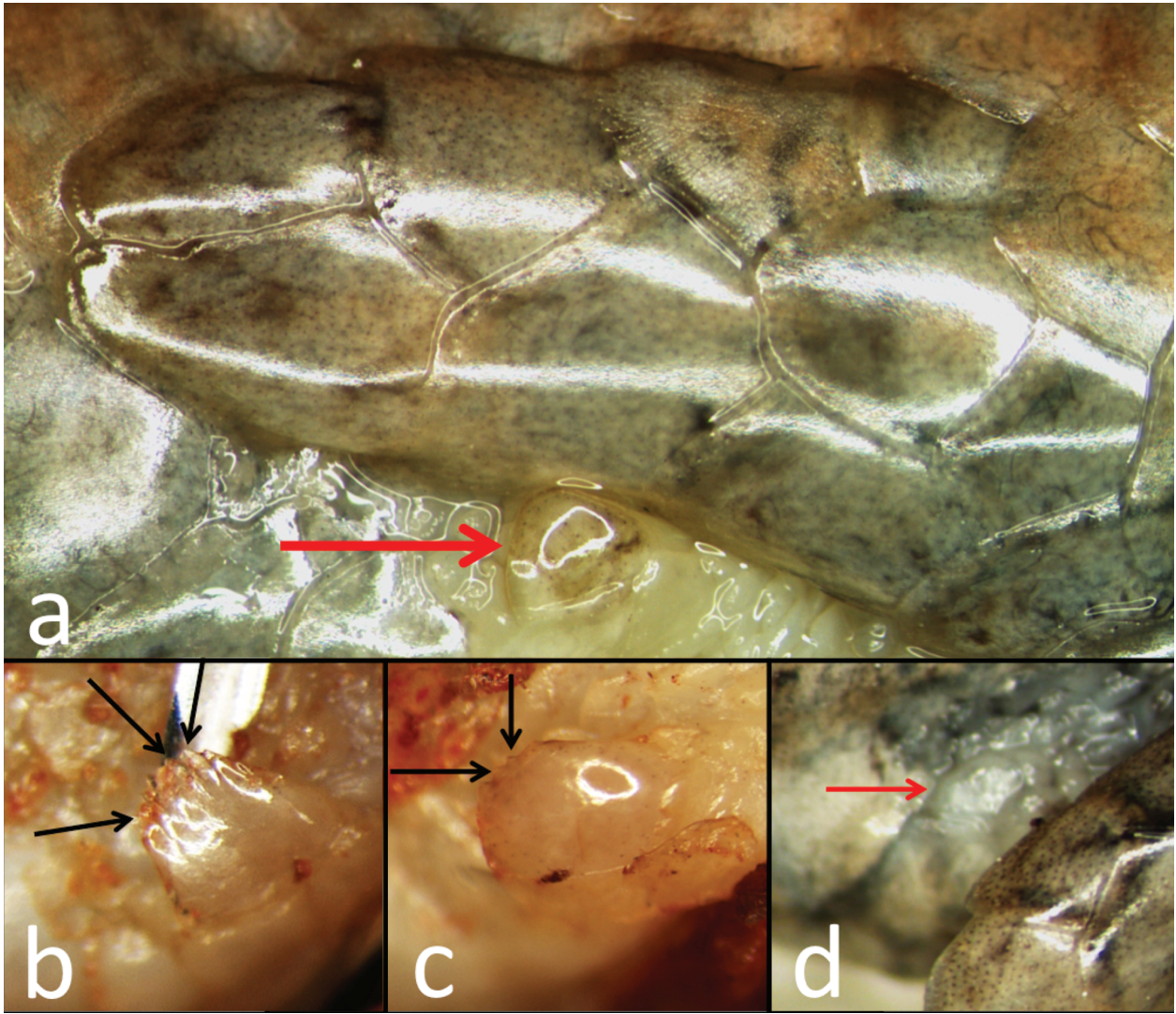


Fig. 4. Lateral view of right cloacal spur of : A) *Delma fraseri* WAMR141191; B) *Delma haroldi* WAMR163615; C) *Delma nasuta* WAMR154288; D) *Delma australis* WAMR140395. Red arrows indicate position of the spur, and black arrows indicate fine projections on posterior-facing distal tip of the spur, a synapomorphy of the *D. butleri* group.

parison to other members of this group, *D. nasuta* exhibits an enigmatic hemipenial condition in which the strongly asymmetrical lobes are both truncate, and the sulcus remains deep and narrow as it terminates at the apex of each lobe. Despite disparate hemipenial morphology, *D. nasuta* has been associated with *D. butleri* and *D. haroldi* based on general morphology (Kluge 1974), osteology (Kluge 1976), and mitochondrial (Jennings et al. 2003) and nuclear DNA (Brennan et al. 2016) results. Here, we also recognize as a synapomorphy of the *D. butleri* clade, the comb-like serrated edge of the cloacal spurs. The spurs are oriented posteriorly, and small projections of the distal spur edge are most pronounced in *D. haroldi*, weaker in *D. butleri*, and less developed but still observable in *D. nasuta* and *D. grayii*. The generalized morphology of the

hemipenes in this *D. butleri* group, as well as in *D. fraseri* and *D. petersoni*, despite a non sister-taxa relationship may suggest that an approximately symmetrical bi-lobed, and relatively unornamented hemipenis may constitute the ancestral *Delma* hemipenial design. Although hemipenial sampling for this genus remains incomplete, this hypothesis would suggest the strongly asymmetrical hemipenis of the northwest Australian group (*D. borea*, *D. desmosa*, *D. elegans*, *D. pax*, *D. tincta*), and the single-lobed hemipenis of the *D. australis* group represent significant morphological divergences.

The monotypic genus *Pletholax* displays a strongly asymmetrical bi-lobed hemipenis which is covered by coarse, dense spines along the lateral and apical portions of the asulcal face, and the sulcal lips. Hemipenes of

Pletholax gracilis are unlike any other pygopodid hemipenis in ornamentation, as well as general shape.

The morphology of *Pygopus* hemipenes is also unique to pygopodids in the presence of a third, undivided, medial lobe. Both observed species, *Pygopus lepidopodus* and *P. nigriceps*, display this medial lobe, as well as differentiated ornamentation of the sulcal and asulcal faces, and ornamented hemipenial bases. Assessment of *Lialis burtonis* is limited to incompletely everted specimens, and as such, makes phylogenetic inference difficult, however, we observe that this species shows a bi-lobed condition, and lobes appear covered in differentiated ornamentation similar to that of *Pygopus*. Similarity in ornamentation may suggest systematic relatedness between *Pygopus* and *Lialis*, or may be an artifact of the much larger adult size of species of these genera, relative to that of other pygopodids. Hemipenial characteristics of *Ophidiocephalus* and *Paradelma* remain unobserved.

Cloacal Spurs and Reproductive Behavior

Morphology of the cloacal spurs and post-cloacal bones and sacs are often mentioned in the context of reproductive biology. In gekkotans, post-cloacal bones and sacs have been reviewed by Kluge (1982), however little attention has been paid to cloacal spurs of geckos. Due to the paucity of behavioral data regarding pygopodids, we draw on the observations of other gekkotans and squamates to address the implication of cloacal spurs and postcloacal bones in reproductive success (Kluge 1982; Kluge 1987). The occurrence of post-cloacal bones is a synapomorphy of gekkotans, uniting the pygopodids with other members of this group (Greer 1989). Although cloacal bones were identified by Kluge (1982) in all examined pygopodid species, cloacal sacs were absent in all *Delma* and *Lialis*; present in both sexes of *Paradelma* and *Pygopus*; present in males of *Aprasia* and *Pletholax*, absent in female *Pletholax*, and inter- and intraspecifically variable in *Aprasia* females.

In the eublepharid gecko *Coleonyx variegatus*, the presence and use of spurs are important for successful mating (Greenberg 1943). Here, after positioning himself alongside the female, and contorting himself to face vent-to-vent, the male slides the closer spur longitudinally along the female's body axis, and across her cloacal opening, in an attempt to gain purchase among the loose skin below the vent. In doing so, the pull of the male's spur draws back the lower lip of the female's cloaca, causing her cloaca to gape, creating an opportunity for the male to evert and insert his hemipenis. Although pygopodid and eublepharid geckos differ morphologically, specifically in the presence or absence of loose post-cloacal skin, spurs may still serve a similar purpose.

In other limb reduced squamates, such as pythonid snakes, cloacal spurs appear as the only external vestige of the hind limbs, where they tip the distal portion of the femur (Greer 1997). The imbricate scales and tighter skin of pythonids more accurately resemble the pygopodid condition, and here male pythons may use the spur to stroke and stimulate the female during courtship, gauge and encourage her receptivity, and as in eublepharids align the cloaca using tactile cues and expedite mating (Greer 1997; Hoser 1985; Murphy et al. 1981; Schouten 1985; Slip & Shine 1988; Walsh 1985). Additionally, spurs may be used in male-male combat, to gain purchase and scratch the opposition (Barker et al. 1979; van der Heijden 1986). It is important to note that the association between spurs and femoral remnants in pythonids, and the presence of external hindlimbs in pygopodids acknowledges the non-homology of spurs across these squamate families. This however, does not require their use to differ.

Cloacal spurs in pygopodids are small structures hidden behind the hindlimb flap, just dorsal and posterior to the cloaca. Spurs are indistinguishable from hindlimb scales in *Aprasia*, but in the comparatively speciose *Delma*, spurs represent another morphological character capable of identifying species groups. As mentioned, the large, comb-like spur of *D. haroldi* (Fig. 4B), is visible to a much reduced degree in the closely related *D. butleri*, *D. nasuta*, and *D. grayii* (Fig. 4C). In *D. australis* and *D. hebesa*, the spur (Fig. 4D) is much less pronounced, rounded, and wider than it is long. In contrast, members of the *D. fraseri* and northwest Australian groups display moderate sized spurs which are rounded in the smaller members of the northwest group *D. borea*, *D. desmosa*, *D. elegans*, *D. pax*, *D. tincta*, and are pointed in *D. fraseri* (Fig. 4A) and *D. petersoni*.

While this study adds to the current knowledge of hemipenial structure and spur morphology and their phylogenetic affinities within Pygopodidae, we present this data as a work in progress. Continued hemipenial assessment of *Delma* species and other pygopodid genera will contribute substantially to our understanding of reproductive evolution and isolation within the flap-footed geckos. The remarkable morphological divergence of pygopodids when compared to their limbed gekkotan ancestors, represents an immense leap, which may present itself in other aspects of anatomy not yet assessed. Complete descriptions of the hemipenes of monotypic *Ophidiocephalus* and *Paradelma*, and more complete description of *Lialis* species may further provide insight into inter-generic relationships within this unique family.

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