



A new seahorse (Teleostei: Syngnathidae: *Hippocampus*) from south-western Australia

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Abstract

A new species of syngnathid fish, *Hippocampus paradoxus* sp. nov., from mid-continental shelf waters of south-western Australia is described from the only known specimen. It can be distinguished from all congeners, including the very similar *H. minotaur* Gomon 1997, by the following features: the lack of a dorsal fin, a series of fleshy, fin-like lobes along the dorsal midline of the trunk and tail and an extremely robust cleithrum and prominent first nuchal plate. In addition to the traditional methods of syngnathid taxonomists, X-ray microtomography (CT scanning) was employed and demonstrated to be a valuable research tool for examining seahorse species that are problematic due to reduced ossification and small size. CT scanning is more capable of imaging poorly ossified and soft tissue regions than traditional radiography and provides a detailed three dimensional view of salient features.

Key words: *Hippocampus paradoxus*, new species, Syngnathidae, X-ray microtomography

Introduction

During a search of the syngnathid holdings of the South Australian Museum (SAMA), an unregistered *Hippocampus* specimen having very unusual characters was discovered. The specimen is clearly similar to *Hippocampus minotaur* Gomon 1997, a small, mid-shelf species known only from several specimens collected in south-eastern Australia. That species is notable for its reduced dermal ossification and in having the lowest number of trunk rings and dorsal fin rays of any previously recognised species of *Hippocampus*. The new specimen is of particular interest as it came from comparable depths off south-western Australia – more than 2000 km west of the known distribution of *H. minotaur* – and, though very similar in general form to the south-eastern species, has some obvious differences. Detailed examination confirmed that although it has meristic and morphological traits previously considered unique to *H. minotaur*, clear distinctions do exist. Attempts to find additional specimens were unsuccessful. This paper provides a name and formal description of the species.

Because of the fleshy nature of the specimen, X-ray microtomography (CT scanning) was employed to examine its skeletal structure. This is a novel technique in seahorse taxonomy as far as we are aware, and the results clearly demonstrate the benefits of using the technique to examine pygmy species that have proven to be problematic using traditional methods.

Methods

The following measurements and counts were made following Lourie (2003), as refined by Lourie & Randall (2003): HL, head length; TrL, trunk length; TaL, tail length; SnL, snout length; OD, orbital diameter; PO, post-orbital length; SnD, snout depth; HD, head depth; PL, length of pectoral fin base; SL, standard length = HL+TrL+TaL; TrR, number of trunk rings; TaR, number of tail rings; PF, number of pectoral fin rays; AF, number of anal fin rays; DF, number of dorsal fin rays.

The fleshy nature of the specimen and reduction or absence of some reference points meant the following measurements could not be made; TD4 trunk depth (from the superior to the inferior trunk ridge) between the 4th and 5th trunk rings; TD9, trunk depth (from the superior to the inferior trunk ridge anterior to the dorsal fin base) between the 9th and 10th trunk rings; DL, length of dorsal fin base; TW, width of trunk (anterior to the dorsal fin base).

CH, coronet height could not be measured by the method recommended by Lourie (2003). The measurement was made instead from the mid cleithral point to the highest point of the fleshy crest.

Most measurements and counts were made under a binocular dissecting microscope, using digital calipers to record measurements to 0.1 mm, and were repeated to ensure accuracy. TrL and TaL were measured from an enlarged photograph incorporating a scale bar, using a wire to follow the curvature of the specimen. Measurements are reported in millimetres and proportional measurements as a percentage of SL, HL or SnL. HL, TrL and TaL are reported as a proportion of SL. SnL, OD, PO, HD and CH are reported as proportions of HL. SnD is reported as a proportion of SnL.

In accord with Vari (1982) and Lourie (2003), a ring is regarded as a segment of a seahorse body *centred* on a transverse ridge (or what remains of it) and overlying a single vertebra. The first trunk ring (TrR1) is centred on the first 'complete' transverse ridge posterior to the cleithrum and overlies the third vertebra. The first tail ring (TaR1) is centred over the first caudal vertebra (identified by the presence of a complete haemal spine).

Transillumination was initially used to determine the number of trunk rings (TrR) and tail rings (TaR) and a radiograph used to verify the counts. The X-ray failed to unambiguously reveal other features of interest, such as possible remnant dorsal fin elements, so X-ray microtomography (CT scanning) was employed to provide a detailed view of osteological features. (SkyScan1076 *in-vivo* microtomograph with scan settings of 42kv, 174µa, zero filter, 1178ms exposure, 0.5 deg rotation step, 9 micron resolution. File conversion and image modelling using SkyScan NRecon, CTan & CTvol software.) The CT scan produced clear images that were, for the most part, easily interpreted (e.g. Fig. 3). For example, whilst the radiograph seemed to indicate the presence of a posteriorly directed haemal spine on the 11th vertebra this feature was clear in the CT scan, confirming the bone and 9th ring to be caudal. Despite the specimen's formalin fixation, some soft tissue features were also clearly discernable, allowing oocytes to be counted and measured.

Institutional abbreviations follow Leviton, *et al.* (1985).

Comparative material *Hippocampus minotaur*: AMS IA.3509, paratype, female (Fig. 2B); AMS IA.3560, paratype, female; NMV A192 holotype, male; NMV A14161, paratype, juvenile.

Systematics

Hippocampus paradoxus sp. nov.

Paradoxical Seahorse

Figs 1, 2A, 3

Hippocampus sp D: Kuitert, 2009:141, 2 figs.

Holotype. SAMA F10490, female, SW of Esperance, Western Australia (approximately 34° 29' S, 121° 32' E), Station GAB 108, depth 102 m, epibenthic sled, RV *Franklin*, collected by S. Hageman, P. Bock & Y. Bone, 26 July 1995.

Diagnosis. A species of *Hippocampus* having: no dorsal fin, a series of fleshy, fin-like lobes along dorsal midline of trunk and tail, 8 trunk rings, 11 pectoral fin rays, extremely robust cleithrum, and prominent first nuchal plate.

Description of holotype. Counts: DF –; PF 11; AF 2; TrR 8; TaR 41.

Measurements: SL 64.6; HL 9.8 (15.2% SL); TrL 14.8 (22.9% SL); TaL 40.0 (61.9% SL); HD 8.4 (85.7% HL); SnL 2.5 (25.5% HL); OD 1.9 (19.4% HL); PO 5.3 (54.1% HL); CH 4.9 (50.0% HL); SnD 1.9 (76.0% SnL); PL 1.2. (1.9% SL).

Head, trunk and tail very compressed, extremely fleshy without obvious bony segments; abdomen, situated between TrR6 and TrR8, broad and protuberant; dorsal surface densely covered with microscopic papillae, papillae also ventrally in some areas, particularly tail, but much less dense; several minute papilliform cirri, visible on expanded supraoccipital.

Head prominent (HL 15.2% SL), axis approximately 90° to trunk axis; cleithrum particularly well developed and robust making head bulbous posteriorly; head deep, (HD 85.7% HL) rising steeply from short snout (SnL 25.5% HL) to a prominent supraoccipital; first nuchal plate also prominent dorsally, of similar height to supraoccipital, relative to horizontal axis of head and much higher than second nuchal plate; coronet only evident in radiograph as diffuse ossification, lying between supraoccipital and first nuchal plate and not in contact with supraoccipital; fleshy ridge-like crest extending posteriorly from supraoccipital, between gill openings, and over first nuchal plate; crest midway between supraoccipital and cleithrum—corresponding to position of coronet—the highest point on head (CH 50.0% HL); gill openings narrowly separated at top of head just anterior to cleithrum; cleithrum prominent laterally and dorsally, reaching to top of first nuchal plate; no cleithral ring evident on dermis.



FIGURE 1. Holotype of *Hippocampus paradoxus* (scale bar = 5 mm).

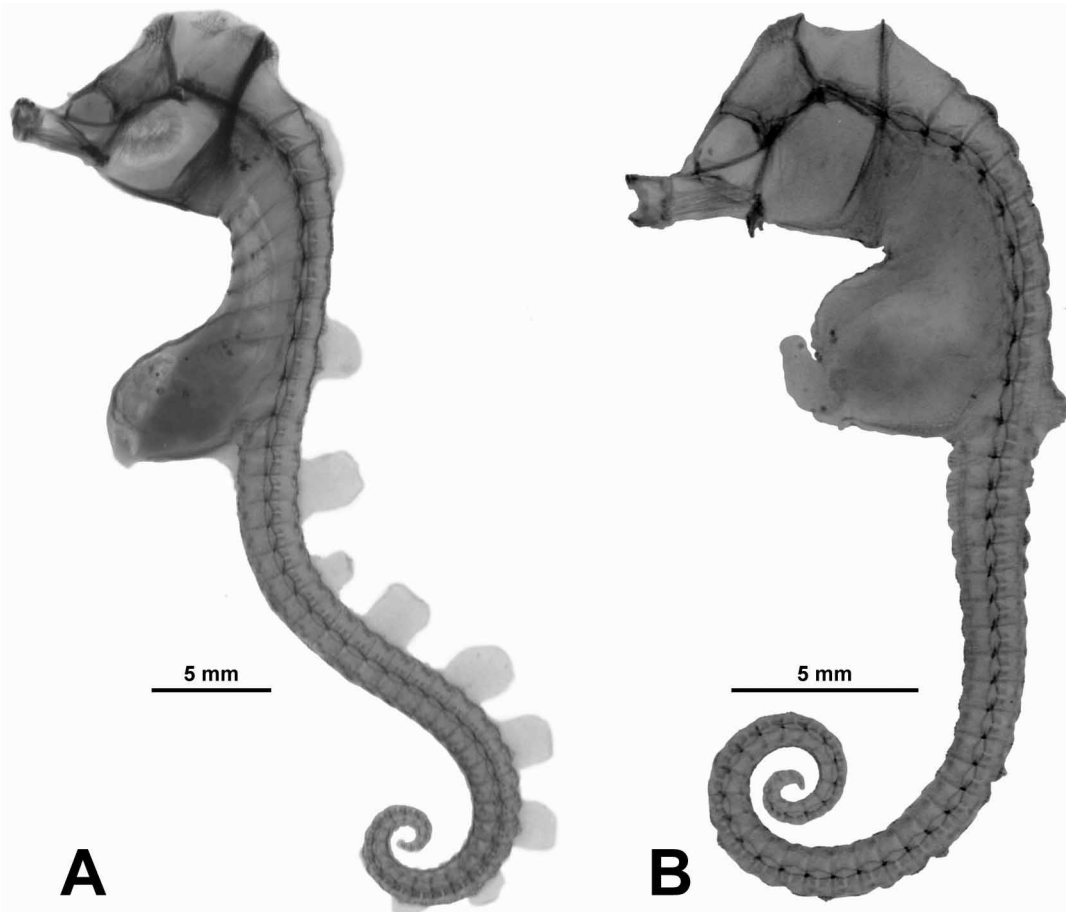


FIGURE 2. Radiographs of *Hippocampus paradoxus* holotype (A) and *H. minotaur* paratype AMS IA.3509 (B).

Orbit surrounded by 9 conical papillae; additional conical papillae on head visible with magnification, some corresponding in position to head spines of species that have them; largest, a broad based ‘nose spine’ just anterior to eyes with smaller papillae on snout and forehead, above eyes, on supraoccipital, on operculum, around gill openings and overlying second nuchal plate; very low circular mound-like tubercle, approximately 1.5mm in diameter, high on head above operculum; blunt fleshy ‘neck spine’, adorned with short cirri, at intersection of superior trunk ridge with TrR1.

Trunk and tail rings barely perceptible but discernable with transillumination; no trunk ridges clearly evident externally, though inferior trunk ridge discernible through dermis; position of superior trunk ridge inferable from cross sectional body shape; caudal segments quadrangular but tail ridges poorly defined.

Dorsal midline of trunk and tail with medially aligned dermal outgrowths (lobes) that superficially resemble fins: 2 on trunk, first on anteriormost 3 trunk rings low, second, covering TrR7 and anterior half of TrR8, resembling dorsal fin but very fleshy and without rays; remaining 10 lobes on tail, more delicate and membranous; base of first on tail covering TaR3, TaR4 and much of TaR5; anterior tail lobes fairly regularly spaced, each spanning about 2–2.5 rings and separated by about 1–1.5 rings; spacing less regular posteriorly, with lobes becoming smaller and less distinctively shaped, posteriormost reduced to small tubercle; second tail lobe considerably smaller than first and third; pairs of small, fleshy, flange-like, lateral outgrowths adjacent to inferior tail ridges ventral to the second and third tail lobes.

Urinogenital opening raised and surrounded radially by pleated skin folds.

Anal fin tiny, fleshy, both rays branching from base; number confirmed by CT scan that shows two pterygiophores at base.

Osteology. CT scan reveals mostly complete, but very light, ossification dorsally on body; superior and lateral trunk ridges clearly discernable but lateral trunk ridge lost with ossification decreasing just prior to tail;

ossification ventral to lateral trunk ridge reduced with trunk rings not fused to neighbours. TrR1 completely encircling ‘neck’ and fused to cleithrum; remaining thoracic rings (TrR2 – TrR6) almost encircling trunk but unfused ventrally and ventral trunk ridge absent; TrR7 similar to anterior rings but because of expanded abdomen not encircling body; ossified ventral elements associated with last trunk ring (TrR8) scaffold-like, somewhat extended and strongly angled posteriorly, following contour of posterior surface of abdomen.

Segmented inferior trunk ridge remnants, originating from TrR6 ossified elements, follow contour of abdomen converging ventro-posteriorly, to run almost parallel adjacent to ventral midline, before diverging after TrR7 and recurving dorso-posteriorly and terminating just anterior to anus, approximately level with origin of tail (see fig 3); cross-shaped ridges on segments the only remnants of intersection with trunk rings posterior to TrR6.

Eleventh vertebra considered to be first caudal vertebra as it is first vertebra with haemal spine; associated 9th ring therefore regarded as first caudal ring; remaining caudal segments totally enclosed by bone, quadrangular.

Based on CT scan, ossified structures at base of second dermal lobe mid-dorsally on trunk (visible in radiograph and initially interpreted as remnants of dorsal fin pterygiophores) appear to be bony outgrowths of superior trunk ridges.

From late 2010, additional CT scan images of *H. paradoxus* can be viewed at www.samuseum.sa.gov.au/ichthyology/research.

Colouration. Colour in life unknown. Colour in preservative yellow-cream, peppered with tiny brown dots; nuchal tubercles densely dotted; series of faint brown spots, some with pale centres, on dorsal midline between TrR3 and TrR6.

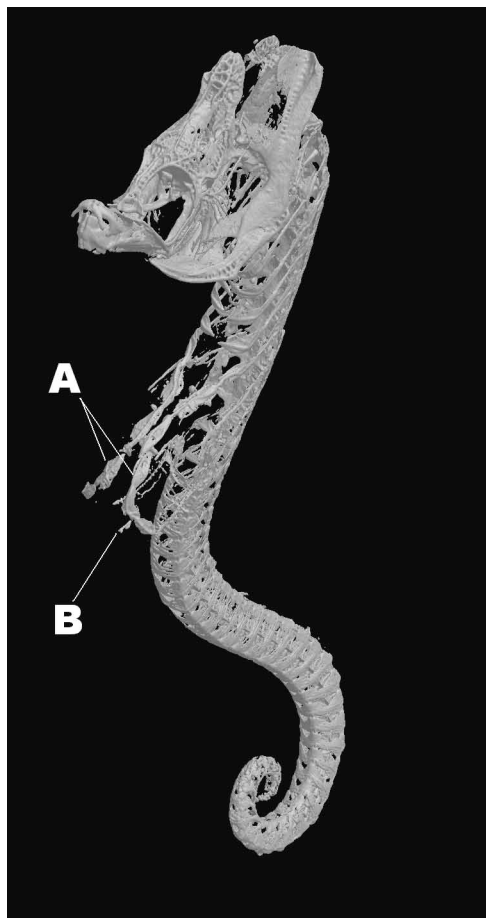


FIGURE 3. Reconstruction of *Hippocampus paradoxus* skeleton from CT scan of holotype. Ventrolateral view, showing inferior trunk ridge remnants (A) and anal fin pterygiophores (B).

Reproduction. The holotype is a female with a greatly distended abdomen holding eleven hydrated oocytes that are each approximately 2 mm in diameter. It was collected in late July. Although male specimens are unavailable, the similarity of this species with *H. minotaur* would support a hypothesis that the pouch is caudal in position.

Habitat. The holotype was collected from the midcontinental shelf benthos (102 m) by researchers targeting bryozoans. High energy waves sweep the sea bed of the Great Australian Bight at this depth producing expanses of rippled sand interspersed with sponge and bryozoan stabilised 'islands' (James *et al.*, 2001). The collection site featured a complex, highly diverse assemblage of bryozoans, including *Adeona*, a number of bushy flexible species (Fam. Catenicellidae), "lace corals" (Fam. Phidoloporidae) and some sponges, on a coarse substrate of calcareous sand (Bock pers. comm.). Seafloor photographs in James *et al.* (2001:561, fig.9) illustrate this habitat type. Water temperatures in the western GAB are moderated by the warm Leeuwin Current during winter. At the time of collection, in late July 1995, the Leeuwin Current was distinct offshore and waters were isothermal (James *et al.*, 2001).

Distribution. Known only from the type locality south west of Esperance, Western Australia, on the extreme western margin of the Great Australian Bight (GAB). There are few records of *Hippocampus* from the more than 2500 km of high energy coastline, stretching from the extreme south west of Western Australia eastward across the GAB to the tip of Eyre Peninsular in South Australia, but the large mid-continental shelf regions have not been extensively collected.

Comparison with similar species. The only species with which *H. paradoxus* is likely to be confused is *H. minotaur*. The two are of similar size and body form and share TrR, TaR, and PF counts. All known specimens of *H. minotaur* have a dorsal fin with 7 or 9 rays (Lourie & Kuiter, 2008) and a raised base on the ultimate trunk ring and first tail ring, whereas *H. paradoxus* entirely lacks a dorsal fin and has no indication of a raised base at this position. The cleithrum of *H. paradoxus* is much more robust and the first nuchal plate is more dorsally prominent with a height about the same as the supraoccipital, relative to the horizontal axis of the head. In *H. minotaur* the supraoccipital ('coronet' of Gomon, 1997) is tallest and the head lacks the fleshy crest and tapers into a much deeper anterior trunk. *Hippocampus minotaur* appears to have lost the thoracic rings ventrally whilst they are retained, in part at least, in *H. paradoxus*. The former also lacks the circumocular papillae of *H. paradoxus*.

Although *H. minotaur* does not feature the dramatic dorsal appendages of *H. paradoxus*, some specimens have mid-dorsal tubercular swellings that may be homologous. These swellings are particularly evident on the paratypes (see Gomon 1997, figs 1b & 3a, Kuiter 2009, p.141). They are situated along the body and tail in nearly the same positions and are very similar in appearance to the distal tubercular swellings on the tail of *H. paradoxus*. Radiography revealed other similarities. The female *H. minotaur* paratype (AMS IA.3509) has ossified structures on the dorsum similar to the bony prominences of the superior trunk ridges evident in the CT scan of *H. paradoxus*. More significantly, the extended TrR8 ossified elements are present and there are indications of inferior trunk ridge remnants girdling the anterior of the abdomen (see fig 2).

Etymology. Latin masc. adj. *paradoxus*, 'strange, contrary to all expectation', in reference to the unusual morphology relative to all other seahorses.

Discussion

Lourie (2003:1) stated "for studies to be repeatable and comparable it is essential that specimens be measured using standard methods". Though few would argue against the adoption of standard methodology, conflicting designations of what constitutes a seahorse trunk and tail ring have appeared in recent literature. Kuiter (2003) and Lourie & Kuiter (2008) reported different trunk and tail ring counts for the type specimens of *Hippocampus colemani* Kuiter. The discrepancy was noted in the latter paper but the reasons for it were not made explicit. The reduction of traditional reference points in atypical taxa, such as *H. colemani*, can make counts difficult but in this example the inconsistency appears to be attributable to differing methodologies. Among recent authors, Vari (1982) and Lourie (2003) followed Ginsburg (1937) and considered rings to be

centred on the raised transverse ridges (or what remains of them) each directly overlying one vertebra. Others, though, appear to view the regions lying *between* the ridges to be rings (e.g., Kuitert 2009, p.15 para.4 & fig.4, p.132 para.5 & annotated X-ray; Horne 2001, p.244 fig.1). From a common origin (e.g. the first complete transverse ridge behind the cleithral ring) the two methods produce counts that differ by one (i.e. n ridges *versus* $n-1$ 'plates'). Furthermore, the point of origin also varies between authors so the methodological anomaly may be masked. Inadequate definition of terms and explanation of methods, an apparent longstanding practice (e.g. see Ginsburg, 1937), increase the inherent potential for confusion and unless elucidated, the differences can pass unrecognised, or 'hybrid' methodologies unwittingly adopted by subsequent researchers. By drawing attention to the problem, we hope to reduce misapprehension as well as reiterate the importance of uniform methods.

Even with the adoption of standard practices, counting trunk and tail rings of pygmy seahorses can present a challenge because of their size and reduced dermal ossification. Vari (1982) showed the trunk rings to be two fewer than the number of precaudal vertebrae in seahorses and taxonomists routinely rely on traditional radiography to make their task easier. X-rays are not always easily interpreted, however, and a two dimensional view is limiting. The use of CT scan images for this study enabled close examination of skeletal structures from any angle and clearly demonstrated the usefulness of the technology in overcoming obstacles. They allowed us to view in detail the pterygiophores, the remnant coronet and the inferior trunk ridge remnants, all of which were obscure in standard radiographs. Microtomography also provides images of poorly ossified and soft tissue regions and enabled us to determine the number of oocytes in the abdomen and to measure them onscreen. With its flexibility and increasing availability, X-ray microtomography is destined to become a very useful tool for fish taxonomists, allowing non-destructive detailed analysis of small and valuable specimens.

Synapomorphies clearly indicate that *H. paradoxus* and *H. minotaur* are sister taxa. Shared TrR and TaR counts alone, separate them from all other seahorse species. Moreover, both are adapted to mid-continental depths, an environment that is extreme for the genus, and they probably favour the same type of habitat. However, their distinctiveness makes determining their relationships to other taxa challenging and has led to a suggestion that *H. minotaur* is not closely related to any other previously described congeners, including other diminutive species having reduced ossification (Lourie & Kuitert, 2008).

Although *H. minotaur* is generally referred to as a 'pygmy seahorse' (e.g. Gomon, 1997; Lourie & Kuitert, 2008), it does not appear to belong to the monophyletic Indo-West Pacific lineage that has been regarded as 'true' pygmy seahorses (Gomon & Kuitert, 2009) since it is not an 'abdominal brooder'. Members of the true pygmy group lack a typical seahorse caudal pouch and brood their eggs and young within the abdomen (Lourie & Randall, 2003), a derived state of taxonomic significance since the placement of eggs has been considered crucial in the systematics of the Syngnathidae (Herald 1959, Wilson *et al.* 2001). Molecular evidence has revealed the true pygmy seahorses to be sister to typical (tail brooding) seahorses (Teske *et al.*, 2004; Hamilton pers. comm.) and the large genetic distance between the two clades suggests an ancient divergence. Although *H. minotaur* is technically a tail brooder, the male seems to have an intermediate brooding condition, with the pouch on the tail pushed anteriorly to such extent that it greatly displaces abdominal organs and has the appearance of being abdominal (Gomon, 1997). A relationship between this state and full abdominal brooding was discounted by Lourie & Randall (2003: 290, para5) based on *H. minotaur* being "more extreme in its differences with respect to the majority of seahorse species (e.g., in the trunk and tail ring, and fin ray counts) than are [the abdominal brooders]". However, as some counts in *H. minotaur* and *H. paradoxus* overlap those of other taxa, including larger sympatric species and true pygmies, and other differences are uninformative the hypothesis cannot yet be rejected. Shared characters, such as a strongly raised cleithral girdle and gill openings at the top of the head, support the notion that the true pygmy and *H. minotaur/paradoxus* lineages may indeed be part of the same radiation.

An alternative hypothesis is that *H. minotaur* and *H. paradoxus* are more closely related to other southern Australian species, including the broadly sympatric *H. bleekeri* Fowler, (of the *H. abdominalis* species complex) and *H. breviceps* Peters. *Hippocampus abdominalis* Lesson and *H. breviceps* constitute the most basal lineage of the tail brooding seahorses included in the molecular phylogeny of Teske *et al.* (2004) which,

with the sister relationship of the true pygmies to the main clade, led to their hypothesis of a West Pacific/Australian origin for seahorses. *Hippocampus abdominalis* (s.l.) and *H. breviceps* have high caudal ring counts like those of *H. minotaur* and *H. paradoxus* but have a generalised seahorse morphology and thus bear no obvious resemblance to the latter two in other respects.

As no molecular data are currently available, we attempted to extract DNA from the holotype of *H. paradoxus* in order to investigate the molecular systematics of the *H. minotaur/paradoxus* lineage. However, the extraction procedure failed to yield high quality DNA, presumably due to the fixation of the specimen in formalin.

Acknowledgements

We are indebted to Peter Blias (SAMA) for X-ray microtomography. Thanks also to Kate Sanders (Univ. Adelaide) for assistance with photography, Sara Lourie (RMMU) for advice on methodology, Healy Hamilton (CAS) for phylogenetic information, Phil Bock (NMV) and Shirley Sorokin (SARDI) for habitat information and Steve Donnellan (SAMA) and two reviewers for comments on the manuscript.

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