

# Revamping Amphinomidae (Annelida: Amphinomida), with the inclusion of *Notopygos*

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This study revises the taxonomic status of the formerly monotypic Archinomidae, which is nested within paraphyletic Amphinomidae according to recent phylogenetic work. We focused our taxonomic sampling to evaluate the affinities of *Notopygos* and genera classified as ‘fusiform’ in body shape, including *Archinome* and *Chloeia*. Prior to this study, the phylogenetic placement of *Notopygos* had not been evaluated. We inferred the phylogenetic relationships of *Notopygos* within Amphinomidae based on nuclear and mitochondrial markers, and cytochrome *c* oxidase subunit I genetic divergences of five *Notopygos* species, including the newly described *Notopygos kekooa* sp. n. from the Gulf of California. The phylogenetic and morphological evidence, now including *Notopygos* species, justified the establishment of two subfamilies within Amphinomidae. In accordance with ICZN Article 36 (Principle of Coordination), both subfamilies are presented as *status novus* in the nomenclature ranks.

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## Introduction

Amphinomidae includes some large, charismatic coral reef-dwelling polychaetes, such as the common ‘fireworms’ *Eurythoe complanata* (Pallas, 1766) (e.g. Barroso *et al.* 2010)

and *Hermodice carunculata* (Pallas, 1766) (e.g. Ahrens *et al.* 2013). Although amphinomids are typically associated with rocky and soft bottoms of intertidal and continental shelf habitats, cryptic taxa are found in deep-sea chemosynthetic

ecosystems (Borda *et al.* 2012, 2013). Wiklund *et al.* (2008) found Archinomidae to be nested within Amphinomidae, rendering the family paraphyletic. Borda *et al.* (2012) corroborated this finding and proposed the division of Amphinomidae into ‘fusiform’ and ‘rectilinear’ clades. The former referring to spindle-shaped amphinoms (Fig. 1), such as *Archinome* and *Chloeia*, and the latter comprised of elongate taxa, including *Amphinome*, *Cryptonome*, *Eurythoe*, *Hermodice*, *Hipponoa*, *Paramphinome* and *Pareurythoe*. The phylogenetic placement of other ‘fusiform’ genera such as *Bathychloeia*, *Chloenopsis*, *Notopygos* and *Parachloeia*, for example, has not been evaluated. *Bathychloeia*, *Chloenopsis* and *Parachloeia* are monotypic and are recorded from difficult to access deep-sea benthic habitats and/or were last collected over a century ago (e.g. McIntosh 1885; Horst 1912). *Notopygos* is the most diverse, with 22 described species (Yáñez-Rivera & Carrera-Parra 2012), and is most notable for sharing morphological features with both *Archinome* and *Chloeia*, including the digitiform branchia (as in *Archinome*) and the ornate trilobed caruncle (as in *Chloeia*), (Kudenov 1991, 1995). In a continued effort to elucidate the biodiversity, systematics and phylogenetic relationships among amphinoms, we evaluated the placement of *Notopygos* within Amphinomidae, described a new species from the Gulf of California (GoC) and revised the taxonomic status of Archinomidae.

## Materials and methods

### Specimen collections and taxon sampling

To evaluate the placement of *Notopygos*, we included the taxonomic sampling from Borda *et al.* (2012) and expanded the sampling of ‘fusiform’ genera with the inclusion of five *Archinome* species (Borda *et al.* 2013), two *Chloeia* species and five *Notopygos* species (see also Table S1). Taxa new to this study include *Notopygos caribea* Yáñez-Rivera & Carrera-Parra, 2012 from Belize and Panama, *Notopygos ornata* Grube, 1856 from the eastern Pacific Ocean (Mexican coast) and GoC, *Notopygos mitsukurii* Izuka, 1910 from

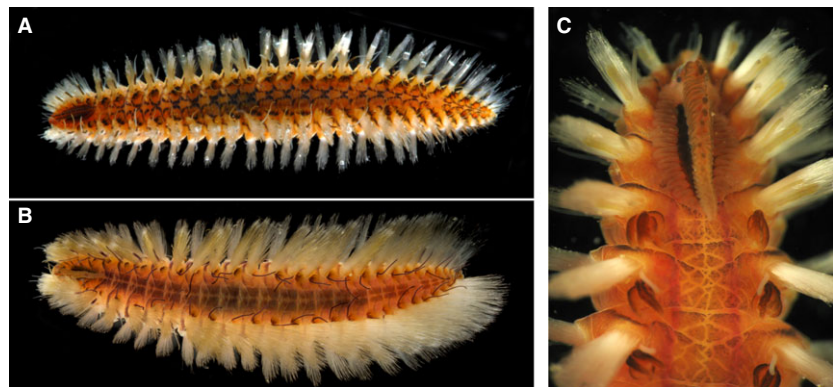
Japan, *Notopygos* sp. from Vanuatu and *Notopygos kekoa* sp. n., described below, from the GoC (data sequence available from paratype only). See also Fig. S1 for a map showing geographic sampling in the Data S1. Amphinomidae was forced to be monophyletic relative to *Euphrosine foliosa* Audouin & Milne-Edwards, 1833 and *Euphrosine armadillo* Sars, 1851 (Amphinomida: Euphrosinidae), which served as outgroup taxa (Wiklund *et al.* 2008; Borda *et al.* 2012). Voucher specimens were deposited in the Scripps Institution of Oceanography Benthic Invertebrates Collection, Florida Museum of Natural History Invertebrate Collection or El Colegio de la Frontera Sur. Collection localities, voucher information, sequence data sources and GenBank accession numbers (KM055006–KM055065, JX028116, JX028117, JX028119, JX028120) are listed in Table S1 (Data S1).

### Morphological evaluation

Evaluation of morphological features for *Notopygos* species included maximum body width (excluding chaetae), body length (prostomium to pygidium), attributes of the branchiae and chaetal lobes. Diagnostic features of the new species were photo-documented and illustrated via line drawings of the caruncle and pigmentation pattern. Semi-permanent slides of chaetae from chaetigers 1, 3, 10 and 15 were prepared to analyse chaetal features. Cephalic terminology follows Orrhage (1990).

### Molecular methods

DNA extraction, PCR amplification and sequencing protocols for nuclear 18S rDNA and 28S rDNA and mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rDNA followed Borda *et al.* (2012, 2013). jMODELTEST 2.1.5 (Darriba *et al.* 2012) was used to infer appropriate evolutionary models for each gene [88 models: COI: TPMuf + I + G; TIM2 + I (*Notopygos* only); 16S: GTR + G; 28S: GTR + I + G; 18S: GTR + I; 24 models: COI: GTR + I + G; GTR + I (*Notopygos* only); 16S:



**Fig. 1** *Notopygos* representatives included in this study. — (A) *Notopygos ornata*, (B) *Notopygos mitsukurii* (Japan) and (C) *Notopygos caribea* (Bocas del Toro, Panama). Image not available for *Notopygos* sp. (Vanuatu). Images provided by CSO (A), Frederik Pleijel (B) and GWR (C).

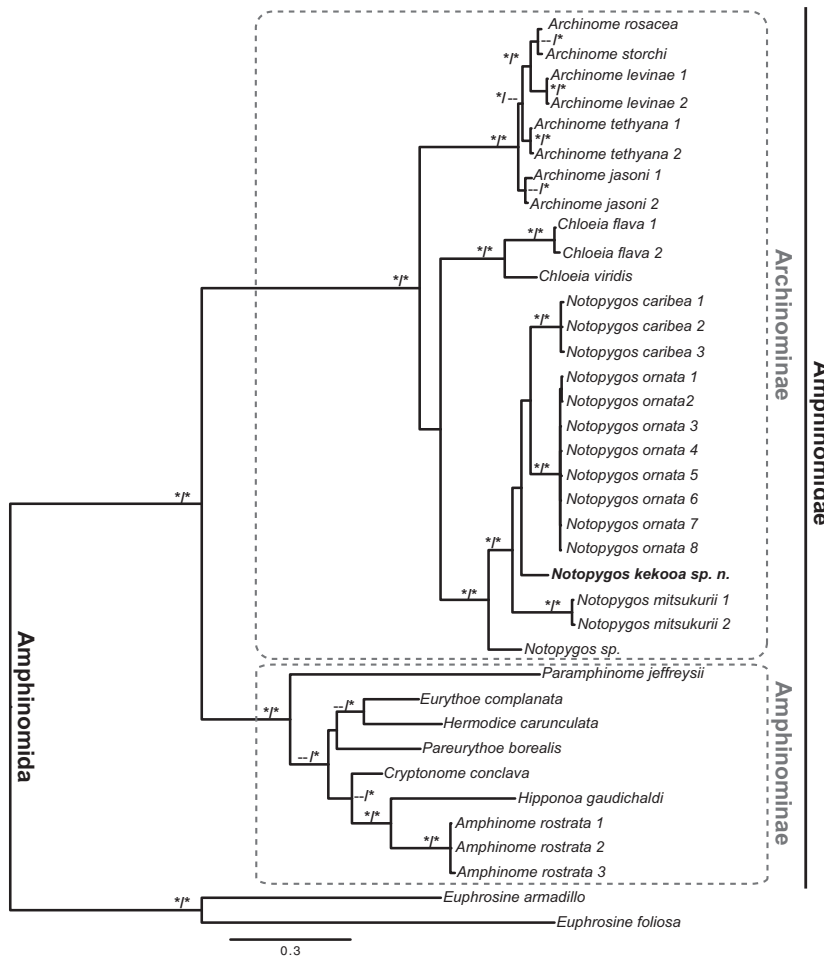
GTR + G; 28S: GTR + I + G; 18S:GTR + I] as selected by the Akaike's information criterion. DAMBE (Xia & Xie 2001) was used to estimate COI saturation via saturation plots of transitions/transversions against GTR-corrected genetic distances and using the Xia *et al.* (2003) saturation test. MEGA 5 (Tamura *et al.* 2011) was used to calculate corrected TrN and uncorrected pairwise COI distances for *Notopygos* species. Phylogenetic relationships and clade support values (bootstrap, boot; posterior probabilities; pp) were estimated under the assumptions of maximum likelihood (boot) and Bayesian inference (pp) as described in Borda *et al.* (2012).

**Results**

**Phylogenetic relationships**

The relationships among 'fusiform' and 'rectilinear' taxa were re-evaluated based on COI (35 sequences; approximately 452 bp, with exclusion of 3rd codon position), 16S (37 sequences; 545 bp), 28S (37 sequences; 970 bp) and 18S (37 sequences; approximately 1736 bp) for a total of

3703 nucleotide characters (Fig. 2). Third codon positions were excluded from combined analyses due to significant transition saturation, which reach a plateau at approximately 26% corrected genetic distance (not shown). The average corrected COI pairwise distance among *Notopygos* species was 31%. *Archinome* + *Chloeia* + *Notopygos* (boot = 100; pp = 1.0) formed a clade that was highly supported as sister (boot = 100; pp = 1.0) to the 'rectilinear' clade (boot = 91; pp = 1.0). *Archinome* (boot = 100; pp = 1.0), *Chloeia* (boot = 100; pp = 1.0) and *Notopygos* (boot = 98; pp = 1.0) were each well supported as clades; however, the recovered sister relationship between *Chloeia* and *Notopygos* was poorly supported (boot < 50; pp < 0.65). Species relationships and clade topologies of *Archinome*, *Chloeia* and 'rectilinear' genera were as recovered by Borda *et al.* (2012, 2013). Inclusion of COI 3rd codon position in combined analyses recovered a similar topology as seen in Fig. 2 (not shown), except for (i) the position of *Archinome leviniae* Borda *et al.* 2013 as sister to the remaining *Archinome* species (see also Borda *et al.* 2013) and (ii) the



**Fig. 2** Phylogenetic hypothesis of Amphinomidae, showing the Archinominae and Amphinomiinae subclades, respectively, and the placement of *Notopygos*. The BI topology shown is based on the combined analyses of cytochrome *c* oxidase subunit I (COI), 16S, 18S and 28S (COI 3rd codon position excluded). Support values at nodes were estimated from the ML bootstrap (boot) and BI posterior probabilities (pp), represented as boot/pp. \*Nodes of high support, boot > 90%; boot > 0.95. Values <90% (boot) and <0.95 (pp) are not shown or indicated by “—”.

unresolved position of *N. kekoa* sp. n. as either sister to *N. ornata* (BI; pp = 0.83) or sister to *N. caribea* + *N. ornata* (ML; boot = 84).

*Notopygos* sp. (Vanuatu) was sister to remaining *Notopygos* representatives, while *N. mitsukurii* was sister (boot < 50; pp = 0.99) to *N. caribea* + *N. ornata* + *N. kekoa* sp. n. (boot < 50; pp < 0.80); interspecific relationships among the latter were poorly resolved. Multiple representatives (Table S1) of *N. caribea* (boot = 100; pp = 1.0) and *N. ornata* (boot = 100; pp = 1.0) clustered within their respective clades. Corrected TrN pairwise COI distances among *Notopygos* species ranged 24–40% (17–25% uncorrected; Table 1).

### Systematics

#### Family Amphinomidae

#### Subfamily Archinominae Kudenov, 1991, *status novus*

*Type genus.* *Archinome* Kudenov, 1991

*Included genera.* *Archinome* Kudenov, 1991, *Chloeia* Savigny in Lamarck, 1818, *Notopygos* Grube, 1855. Provisionally included until further study: *Bathychloeia* Horst, 1912; *Chloenopsis* McIntosh, 1885, and *Parachloeia* Horst, 1912.

*Diagnosis.* Trilobate caruncle, with narrow and smooth lobes (Fig. 3A) or median plicate lobe with flat lateral plicate plates (Figs 3B and 4B,I). Branchiae ramified from single stem, bipinnate (Fig. 3G) or comprised of digitiform rami (Fig. 3H). Dorsal cirri with cirrophore (Fig. 3F–H). Accessory dorsal cirri, without cirrophore, present only on anterior chaetigers (Fig. 3F,G) or present on all chaetigers (Figs 1A,B and 4A). Anus position, dorsal on posterior chaetigers (Fig. 4K) or (dorso)terminal on last chaetiger (see also Borda et al. 2012).

*Remarks.* A fusiform body shape is seen in several amphinomid genera, although most have not been thoroughly evaluated phylogenetically, warranting further study. The accessory dorsal cirrus (Yáñez-Rivera & Carrera-Parra 2012), also referred to as dorsal cirrus (Kudenov 1991) or

**Table 1** Corrected (TrN; below diagonal) and uncorrected (italics; above diagonal) pairwise genetic distances among *Notopygos* species; intraspecific TrN genetic distances (bold, italics) are found along the diagonal

	I.	II.	III.	IV.	V.
I. <i>Notopygos caribea</i>	<b>0.008</b>	0.184	0.207	0.197	0.195
II. <i>Notopygos kekoa</i> sp. n.	0.260	–	0.187	0.173	0.207
III. <i>Notopygos mitsukurii</i>	0.300	0.272	<b>0.004</b>	0.220	0.205
IV. <i>Notopygos ornata</i>	0.279	0.240	0.327	<b>0.011</b>	0.242
V. <i>Notopygos</i> sp. (Vanuatu)	0.285	0.304	0.300	0.395	–

(median) branchial cirrus (Horst 1912; Barroso & Paiva 2011), is lacking in Amphinominae (established below), but is present in *Bathychloeia*, *Chloenopsis* and *Parachloeia* (J. D. Kudenov, personal observation). We differentiate the accessory dorsal cirrus from the true dorsal cirrus (=lateral; Kudenov 1991, 1993, 1995) in lacking a cirriphore and the variable position relative to branchiae and notochaetal lobe. The dorsal cirrus is surrounded by notochaetae and is located on the edge of the notopodia as in other amphinomids (Fig. 3G–I). Branchiae can be bipinnate, as in *Chloeia*, or a tuft comprised of digitiform rami, as in *Archinome* and *Notopygos*. Digitiform branchial tuft arising from a single stem is also shared with members of Amphinominae. The dorsal position of the anus on posterior segments is unique to *Notopygos* and *Archinome*, while the (dorso)terminal position of the anus of *Chloeia* is shared with Amphinominae.

Based on the phylogenetic evidence (Wiklund et al. 2008; Borda et al. 2012; present study) and evaluation of key morphological features, we here establish Archinominae for the clade that includes genera with an accessory dorsal cirrus (i.e. *Archinome*, *Chloeia* and *Notopygos*), and provisionally includes other ‘fusiform’ genera, until further re-evaluation (i.e. *Bathychloeia*, *Bathynotopygos*, *Chloenopsis* and *Parachloeia*). Although fusiform body shape is consistent with members of this clade, several Amphinominae genera and their juvenile forms also exhibit ‘fusiform’ body shapes (B. Yáñez-Rivera, personal observation). Therefore, we restrict the use of body shape to genus- and species-level descriptions, unless evidence from combined genetic and morphological evaluation in future work shows that this feature is not convergent among amphinomids.

#### *Genus Notopygos* Grube, 1855

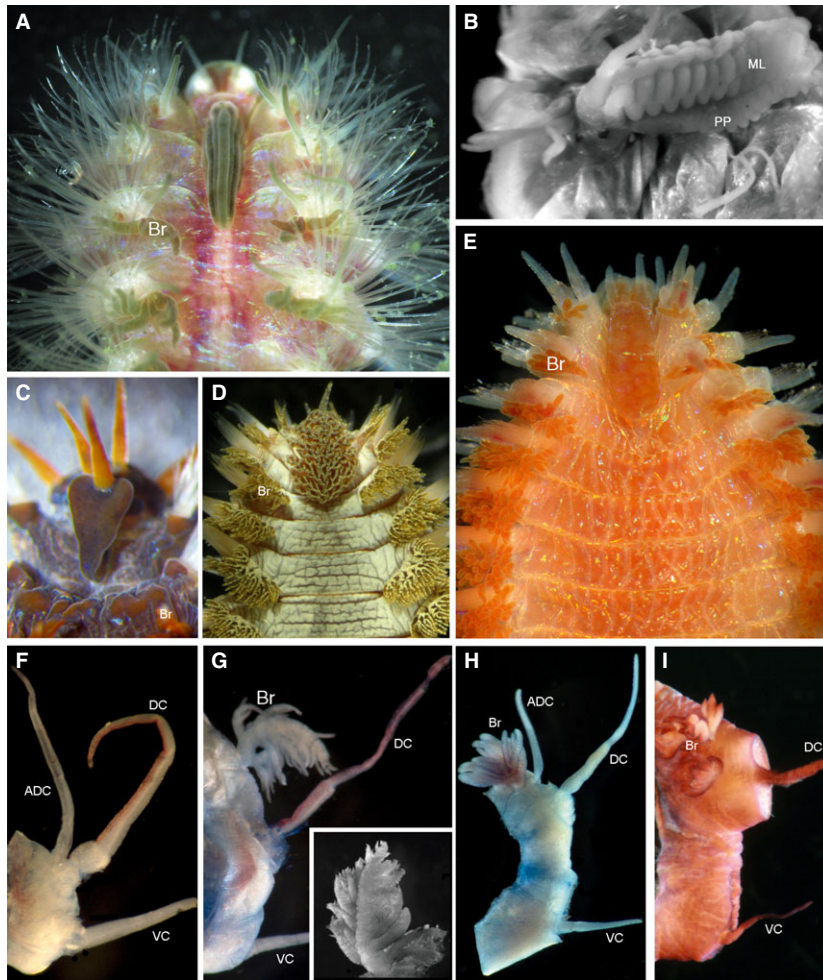
*Type species.* *Notopygos crinita* Grube, 1855

*Diagnosis.* Fusiform body shape. Trilobate caruncle, with plicate median lobe and flat lateral plicate plate (Fig. 4B,I). Dorsal pigmentation patterns variable. Branchial tuft arising from single stem, comprised of digitiform rami (Figs 3H and 4C). Dorsal cirri with cirrophore (Fig. 4D). Accessory dorsal cirri without cirrophore, present on all chaetigers (Fig. 4A, H). Anus position, dorsal on posterior chaetigers (Fig. 4A).

*Included species.* See Data S1 for complete list of *Notopygos* species (text and Fig. S1).

#### *Notopygos kekoa* sp. n. (Fig. 4)

*Type material.* Holotype SIO-BIC-2464, Angel de la Guarda, Baja California, Mexico, 29°33'N, 113°33'W, 06 July 2009, <5 m, rocky reefs under rocks. Paratype SIO-BIC-2274, Angel de la Guarda, Baja California, Mexico 29°33'N, 113°33'W, 06 July 2009, <5 m, rocky reefs under rocks.



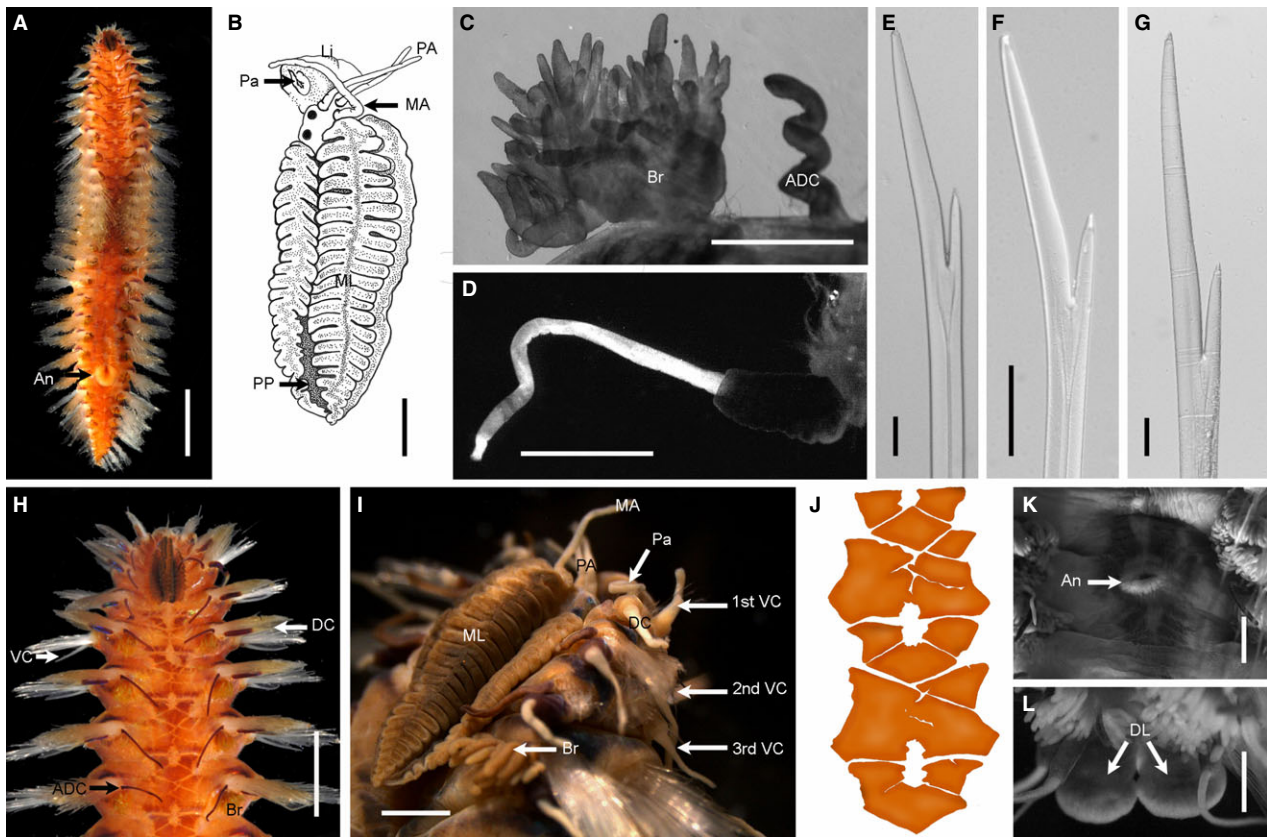
**Fig. 3** Morphological attributes of Amphinomidae. — (A) Narrow elongate trilobed caruncle: *Archinome levinae*, (B) trilobed caruncle with plicate plate: *Chloeia australis*, (C) heart-shaped caruncle: *Amphinome rostrata*, (D) caruncle with convergent folds: *Hermodice carunculata*, (E) caruncle: *Eurythoe complanata*, (F) parapodium from first chaetiger with accessory dorsal cirri: *Chloeia viridis*, (G) parapodium from chaetiger 10 with bipinnate branchiae: *Chloeia viridis* (note: accessory dorsal cirrus is absent from chaetiger 4); insert, bipinnate branchiae: *Chloeia pinnata*, (H) parapodium from chaetiger 10 with branchial tuft and accessory dorsal cirrus: *Notopygos ornata*, (I) parapodium from chaetiger 15 with branchial tufts and with accessory dorsal cirrus absent: *Hermodice carunculata*. Images provided by GWR (A, D, E) and BYR (B, C, F–I). ADC, accessory dorsal cirrus; Br, branchia; DC, dorsal cirrus; ML, median (plicate) lobe; PP, plicate plate; VC, ventral cirrus.

**Description.** *Holotype*: mature female, complete with 36 chaetigers, 6.5 cm long and 1.3 cm wide. Body fusiform, live colour pattern ranging from orange to brown with yellow–orange branchiae and dark pigmented caruncle (Fig. 4A,H). Accessory dorsal cirri and cirrophore of dorsal cirri deep violet (Fig. 4H). Complex pigmentation with trapezoidal and rhomboidal forms covering dorsum (Fig. 4H,J). Prostomium rounded with two pairs of eyes, similar size (Fig. 4B). Median antenna arising from posterior margin of prostomium (Fig. 4B,I). Paired antennae arising from anterior prostomial margin (Fig. 4B,I). All antennae equal length (1.5 mm long). Buccal lips extending from prostomium and reflect ventrally. Palps laterally directed on buccal lips, shorter than antennae (1.1 mm), damaged. Mouth ventral on chaetiger 3.

Trilobed caruncle (4 mm long, 1.8 mm wide). Median plicate lobe elevated with approximately 22 bilateral folds (Fig. 4B,I). Flat lateral plicate plates each with approximately 21 folds (Fig. 4B). Branchial tufts comprised

of digitiform rami, first appearance on chaetiger 4 (Fig. 4C,I), present to pygidium. Each branchia with short stem, branching in several digitiform filaments of variable thickness and length. First branchia with approximately 21 filaments, second branchia with approximately 50 filaments and mid-body branchiae with more than 100 filaments, decreases in number posteriorly.

Parapodia biramous, notopodia with accessory and dorsal cirri and neuropodia with single ventral cirrus. Accessory dorsal cirri, deep violet, similar length throughout body ( $2 \pm 0.2$  mm, Fig. 4H). Dorsal cirri with short deep violet cirrophores (0.5 mm long) and slender unpigmented cirrostyles (1.7 mm long) in first chaetigers, subsequent chaetigers, after 5, with longer cirrophores (1.0 mm) and cirrostyles (3.2 mm, Fig. 4D). Ventral cirri with short unpigmented cirrophores (0.2 mm long) and cirrostyles from 1.3 to 2.5 mm long. Cirrophore on second neuropodium 0.5 mm long. All chaetae asymmetrically furcate, smooth. Proportion between tines varies from 1:3 to 1:4



**Fig. 4** *Notopygos kekooa* sp. n. — (A) Live specimen complete, dorsal view, (B) Caruncle illustration, (C) Branchiae and accessory cirrus from chaetiger 15, (D) Dorsal cirrus from chaetiger 15, (E) Notochaeta from chaetiger 3, (F) Neurochaeta from chaetiger 3, (G) Neurochaeta from chaetiger 10, (H) Anterior end of live specimen, dorsal view, (I) Anterior end lateral oblique view, (J) Pigmentation pattern, (K) Anus, dorsal view, (L) distal lobes. Scale bars: A, 1 cm; B–D; I, 1 mm; E–G, 50  $\mu$ m; H, 5 mm; K–L, 0.5 mm. Images and illustrations by BYR and GMO, CSO provided (A) and (H). ADC, accessory dorsal cirrus; An, anus; Br, branchia; DC, dorsal cirrus; DL, distal lobes (on last chaetiger); MA, median antenna; ML, median (plicate) lobe; Pa, palps; PA, paired antennae; PP, plicate plate; VC, ventral cirrus.

(Fig. 4E–G). Notochaetae from first chaetigers greater in length and width than neurochaeta, subsequently chaetae similar in size. Anus dorsal, located on chaetiger 29 (Fig. 4K). Posterior end margin with a pair of distal lobes (0.8 mm long, 0.8 mm wide; Fig. 4L).

**Gametes.** Gametes present within coelom, entire length of body. Oocytes 99–119  $\mu$ m in diameter (mean:  $108 \pm 5.5 \mu$ m,  $n = 30$ ).

**Etymology.** The specific name refers to an adjective form derived from the kiliwa word ‘kekoo’ meaning woman and refers to the holotype being a mature female. The kiliwa is an ancient language from a native community from northern Baja California (Estrada & Farldow 2004).

**Distribution and abundance.** Angel de la Guarda, Gulf of California, Mexico. This species appears to be rare. In two scientific expeditions in Gulf of California Islands (2009,

2010) with more than 100 dives, only two specimens were collected.

**Remarks.** *Notopygos kekooa* sp. n. is distinguished from other *Notopygos* species on the basis of its complex pigmentation pattern consisting of rhomboidal and trapezoidal forms (Fig. 4H,J) and also supported as distinct by the molecular data. *Notopygos kekooa* sp. n. shares the first appearance of branchiae starting on chaetiger four and an overlapping distribution within the GoC with *N. ornata* (see Table 2). In both species, the dorsal pigmentation pattern is complex, differs in the percentage cover and form. *Notopygos ornata* shows triangular and rhomboid forms in a symmetrical pattern with 50% coverage, while in *N. kekooa* sp. n., trapezoidal forms are predominant and the percentage cover is greater (80%). In addition, the former species has circular projections on the crest of the median lobe of the caruncle, similar to *N. caribea* (Fig. 1C), while the species described here lacks these projections (Fig. 4B,I).

**Table 2** Traditional diagnostic characters for *Notopygos* species. \*indicates characterization of anus position after the original description

Species	1st appearance of branchiae	Anus position	References
<i>N. albiseta</i>	Unspecified	22/23	Holly (1939)
<i>N. andrewsi</i>	5	24	Monro (1924)
<i>N. caribea</i>	5	23	Yáñez-Rivera & Carrera-Parra (2012)
<i>N. cirratus</i>	5	23/24	Horst (1911)
<i>N. crinita</i>	5	21*	Grube (1855), Ehlers (1887)
<i>N. flavus</i>	5	Unspecified	Haswell (1878)
<i>N. gardineri</i>	Unspecified	24	Potts (1909)
<i>N. gigas</i>	5	25	Horst (1911)
<i>N. gregoryi</i>	Unspecified	28/29	Holly (1939)
<i>N. hispidus</i>	6	21	Potts (1909)
<i>N. horsti</i>	5	22/23	Monro (1924)
<i>N. kekoos</i> sp. n.	4	29	Here
<i>N. labiatus</i>	5*	Unspecified	McIntosh (1885), Horst (1911)
<i>N. megalops</i>	6	18*	McIntosh (1885), Yáñez-Rivera & Carrera-Parra (2012)
<i>N. mitsukurii</i>	5	26	Izuka (1910)
<i>N. ornata</i>	4	24*	Grube & Ørsted in Grube (1857), Monro (1933)
<i>N. parvus</i>	3	Unspecified	Haswell (1878)
<i>N. rayneri</i>	5	22	Baird (1870)
<i>N. sibogae</i>	5	23	Horst (1911)
<i>N. splendens</i>	Unspecified	Unspecified	Kinberg (1857)
<i>N. subpragigas</i>	5	29	Uschakov & Wu (1962)
<i>N. variabilis</i>	5	22–25	Potts (1909)

The anus position on chaetiger 29 (Fig. 4A) is a feature shared with *N. subpragigas*, but they differ in the start of the branchiae (see Table 2).

### Subfamily Amphinominae Savigny in Lamarck, 1818, *status novus*

*Type genus.* *Amphinome* Brugière, 1789

*Included genera.* *Amphinome* Brugière, 1789, *Cryptonome* Borda et al., 2012, *Eurythoe* Kinberg, 1957, *Hermodice* Kinberg, 1957, *Hipponoa* Audouin & Milne-Edwards, 1830, *Paraerythoe* Gustafson, 1930 and *Paramphinome* Sars, 1869. Provisionally included until further study: *Benthoscolex* Horst, 1912, *Branchamphinome* Hartman, 1967, *Linopherus* Quatrefages, 1865, and *Pherecardia* Horst, 1886.

*Diagnosis.* Caruncle variable (Fig. 3C–E). Branchial tuft from single or double stems (Fig. 3I), comprised of digitiform rami (Fig. 3E). Dorsal cirri and ventral cirri present. Accessory dorsal cirri absent. Anus position (dorso)terminal on last chaetiger.

*Remarks.* Based on the phylogenetic evidence (Wiklund et al. 2008; Borda et al. 2012; present study) and evaluation

of key morphological features (Borda et al. 2012; present study; J. D. Kudenov, personal observation), we here use the name Amphinominae for the clade that includes genera that lack the accessory dorsal cirrus. Although rectilinear body shape was characterized by Borda et al. (2012) to be associated with members of this clade, several genera, namely *Benthoscolex* and *Branchamphinome*, and juvenile stages of amphinomids exhibit ‘fusiform’ bodies (B. Yáñez-Rivera, personal observation). Therefore, we restrict the use of body shape to genus- and species-level descriptions, unless evidence from combined genetic and morphological evaluation in future work shows that the ‘rectilinear’ body shape is a fixed synapomorphy among members of this clade.

### Discussion

#### *Taxonomic status of Archinomidae and Amphinomidae*

Archinomidae, and the genus *Archinome*, were established by Kudenov (1991) because *Euphrosine rosacea* Blake, 1985 (originally placed in Euphrosinidae due to the similarity in shape and form of the caruncle) exhibited a mixture of diagnostic features from both Amphinomida families, Amphinomidae and Euphrosinidae. This species was removed from Euphrosinidae due to lacking the branchial arrangements in transverse rows and lacking ringent chaetae, typical of *Euphrosine* species. *Archinome*/Archinomidae is now recognized as a lineage within Amphinomidae (Wiklund et al. 2008; Borda et al. 2012), and the inclusion of *Notopygos* species confirms their membership within the ‘fusiform’ clade as proposed by Borda et al. (2012). According to Article 36, Principle of Coordination Article (ICZN 1999), each clade can be considered a subfamily and classified as *status novus*, when any rank in the family group formed from the stem of the name of the type genus, with appropriate change of suffix, and has the same authorship and date. Therefore, we propose that Borda et al.’s (2012) ‘fusiform’ (Clade I) and ‘rectilinear’ (Clade II) clades be designated as subfamilies of Amphinomidae, Archinominae and Amphinominae, respectively (Fig. 2). The genera evaluated to date group within reciprocally monophyletic clades and are morphologically distinguished based on the presence (Archinominae) or absence (Amphinominae) of the accessory dorsal cirrus (Fig. 3F,H). We here delineate Archinominae as a clade that includes *Archinome*, *Chloeia* and *Notopygos*, and Amphinominae a clade that currently includes *Amphinome*, *Cryptonome*, *Eurythoe*, *Hermodice*, *Hipponoa*, *Paramphinome* and *Pareurythoe*. Evaluation of the morphology and phylogenetic placement of other unrepresented ‘fusiform’ (e.g. *Bathychloeia*, *Chloenopsis* and *Parachloeia*) and ‘rectilinear’ (e.g. *Linopherus* and *Pherecardia*) genera aligns with the diagnoses provided above for both subfamilies and will be addressed elsewhere.

### Phylogenetic affinities of *Notopygos*

Kudenov (1991, 1995) classified *Notopygos* as part of a '*Chloeia-Notopygos* species complex' within Amphinomidae, consistent with Gustafson (1930). While the idea of a 'species complex' was, in retrospect, a vague formulation, the discovery of *Archinome* provided new insights that questioned the monophyly of Amphinomidae, based on morphology. The three genera were considered allied in part by having similarly positioned accessory dorsal cirri and branchia. *Notopygos* shares a prominent and ornamented trilobed caruncle, as in *Chloeia* (Fig. 3B), and both digitiform branchiae (Fig. 3A) and a dorsal anal position, as in *Archinome* (terminal in *Chloeia*). Only recently, a close relationship between *Chloeia* and *Archinome* was shown on the basis of molecular evidence (Wiklund *et al.* 2008; Borda *et al.* 2012), and now, the inclusion of *Notopygos* corroborates its placement within Archinominae. Although the phylogenetic hypothesis did not indicate high support for a '*Chloeia-Notopygos* species complex', it did support the monophyly of each genus and provided support for their evolutionary affinities.

### Taxonomy of *Notopygos*

Little has changed since Potts (1909) discussed the limited systematic value of some diagnostic features used to distinguish *Notopygos* species. For example, the segments of the first appearance of branchiae and the position of the dorsal anus were considered traditional morphological features (Horst 1911; Yáñez-Rivera & Carrera-Parra 2012). However, at least half of the known species share the same segment of the first appearance of the branchiae in segment 5 (Table 2). Similarly, evaluation of the morphology of *Archinome*, where the position of the anus was formally considered to be diagnostic (Fiege & Bock 2009), was shown to not be a fixed feature among and within species, but rather size dependent of the specimen (Borda *et al.* 2013). Available evidence in *Notopygos* suggests that the position of the dorsal anus shows low variation (Monro 1933). Nevertheless, this feature alone may be unusable for diagnosis due to some species sharing the anus position or this information left unspecified in others (Table 2).

Poor taxonomic knowledge and the lack of a standardized taxonomy for *Notopygos* have contributed to misidentification and questionable species records, thus confounding biodiversity estimates. *Notopygos crinita*, for example, described from St. Helena Island, in the South Atlantic Ocean, is reported as widespread in the western Atlantic (Yáñez-Rivera & Carrera-Parra 2012) and documented as an invasive in the Mediterranean (Occhipinti-Ambrogi *et al.* 2011). While this species is described as lacking any dorsal pigmentation pattern (Grube 1855), the presence of an accessory dorsal cirrus was not mentioned and the first branchiae appear on chaetiger 5 (Yáñez-Rivera 2009);

species records beyond the type locality remain ambiguous for this taxon.

Morphological features, including dorsal pigmentation pattern (live and after preservation) and aspects of the caruncle, were extensively documented in this study. We consider pigmentation patterns to be an important diagnostic feature that appears to be discrete among species (e.g. Yáñez-Rivera & Carrera-Parra 2012; Figs 1 and 4H,J); however, their description can be subjective and confusing without proper illustration and/or photo documentation prior to fixation. As specimens tend to lose their pigmentation after preservation, gross morphological features that are left for diagnosis tend not to be species specific. Thus, evaluation of morphology alone for *Notopygos* is of limited value without live documentation of dorsal pigmentation patterns and the inclusion of genetic data analyses (see also Yáñez-Rivera & Carrera-Parra 2012).

### Conclusions

We continue to advocate that molecular data be evaluated simultaneously with morphology for improved taxonomic diagnoses, with the incorporation of documentation of pigmentation pattern, as well as of the integration of ecological and reproductive data when possible. The use of pigmentation pattern to distinguish *Notopygos* species is supported by molecular data. The lack of a standardized taxonomy and understanding of the phylogenetic relationships of Amphinomidae have led to confusion in other amphinomid taxa, including *Amphinome* (Borda *et al.* 2012), *Chloeia* (Barroso & Paiva 2011) and *Archinome* species (Borda *et al.* 2013). We consider this study a step forward to begin shedding light on *Notopygos* and a continuing contribution towards clarifying Amphinomida systematics.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Type localities of *Notopygos* species (red symbols), and collection localities of *Notopygos* specimens included in this study (black symbols).

**Table S1.** Taxa and new sequences included in this study, with collection locality or sequence source, voucher information and GenBank accession numbers.

**Data S1.** Results.