RESEARCH ARTICLE



Terrestrial molluscs of Pemba Island, Zanzibar, Tanzania, and its status as an "oceanic" island

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Abstract

Pemba is thought to have had a longer and/or stronger history of isolation than its better-known counterpart, Unguja. The extent to which the biota support this hypothesis of greater oceanicity have been debated. Here, Pemba's terrestrial mollusc ("land-snail") fauna is surveyed and reviewed for the first time. We find at best equivocal evidence for the following hallmarks of greater oceanicity: impoverishment, imbalance, and a high rate of endemism. At least 49 species are present, families are represented in typical proportions, and there are only between two and four island-endemic species - i.e. a 4% to 8% rate of endemism. For land-snails, isolation thus seems to have been short (Pleistocene) or, if longer, weak. Nevertheless, Pemba does host endemic and globally rare species. Forty-five percent of the species found, including most of these, are restricted to forest reserves, with Ngezi Forest Reserve particularly rich. A further 45% are able to tolerate the island's woody cultivated habitats. One new snail species (Cyclophoridae: *Cyathopoma*) and one new slug species (Urocyclidae: *Dendrolimax* pro tem.) are described. New data and illustrations are provided for other taxa.

Keywords

Land-snails, Stylommatophora, Pulmonata, Caenogastropoda, Mollusca, Cyathopoma, Dendrolimax, snails, slugs, endemism, Pemba

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Introduction

Pemba is one of the two main Indian Ocean islands of Zanzibar, Tanzania, the other being Unguja (itself commonly referred to as "Zanzibar"). It has long been recognised that although the two are of comparable size, topography, distance from the mainland, as well as climate and climatic history (e.g. Clarke 2000), they differ in their geological and biotic history. Geologically, both islands consist of Miocene rocks of continental origin fringed by uplifted Pleistocene coral rag limestone platforms (Kent et al. 1971, Schluter 1997, Mukuza et al. 2002). However, the channel separating Pemba from the mainland and from Unguja reaches approximately 800m depth while that between Unguja and the mainland is less than 200m; Pemba effectively lies off the continental shelf (Fig. 1A). While the presence of a terrestrial fauna in Pemban rocks supports the presence of land since at least the middle Miocene (Pickford 2008), this land is believed to have been part of the mainland until the formation of the Pemba Channel by a graben fault approximately 6 Ma (early Pliocene; Kent et al. 1971, Clarke and Burgess 2000). Other geological evidence has suggested an even earlier separation in the late Miocene (see Moreau and Pakenham 1940, Stockley 1942, Pickford 2008). Sea level lowstands of up to 145m below present since Pemba's separation, while critical for the evolution of other western Indian Ocean island faunas (e.g. Peake 1972, Warren et al. 2010), would therefore not have sufficed to reconnect Pemba to the mainland or Unguja. Unguja, in contrast, was most recently isolated from the mainland in the Pleistocene (Stockley 1942, Clarke and Burgess 2000) probably as little as 10-18 thousand years ago. Thus even if Pemba's isolation was as recent as the latest Pliocene (1 Ma), it would have remained an island for up to 100 times longer than Unguja.

A corollary of the theory of island biogeography is that the hallmarks of greater "oceanicity" (= isolation, sensu Peake [1972]) would then be expected in Pemba's biota relative to that of Unguja: a depauperate fauna skewed towards vagile species and with a greater degree of endemism. Which of these features the fauna (which is imperfectly known and certainly modified by man) shows has been the subject of debate. The fauna of both islands, plus that of the Tanzanian island of Mafia, is on the whole derived from the adjacent mainland (e.g. Voeltzkow 1923, Moreau and Pakenham 1940, Burgess et al. 1998). Pemba was found to be an important sub-centre of endemism for the region by Burgess et al. (1998), the only one of the three islands to be so, largely as a result of high faunal endemism. As Beentje (1990) and Baker and Baker (2002) suspected, further endemics have been discovered in other groups, sometimes reported to have a Mascarene affinity (e.g. Nahonyo et al. 2005, Dijkstra et al. 2007). Addressing the vertebrate fauna of the islands, Moreau and Pakenham (1940) noted that Pemba's fauna was particularly impoverished and unbalanced, and showed Mascarene affinities not present in Unguja. However, they described endemism as low and noted that these "peculiarities" of Pemba were shown mainly by volant species. They probably had in mind the endemic Pemba Flying Fox Pteropus voeltzkowi, Pemba Scops-owl Otus pembaensis, Pemba White-eye Zosterops vaughani, Pemba Sunbird Nectarinia pembae and perhaps the Pemba Green Pigeon Treron pembaensis. Peake (1971) gave both Pteropus and Otus



Figure 1.A Pemba and the surrounding area. Contours: 200m and 1000m (above sea level); 200m (below sea level). The land below 400m, including the islands, roughly corresponds to the Zanzibar-Inhambane vegetation mosaic of White (1983) in which coastal forest fragments are scattered. **B** sites surveyed on Pemba in 2009, numbered as in Table 1, with forest reserves (shaded areas) and large towns marked.

as examples of western Indian Ocean island lineages found only upon islands even where they were close to continents, whose presence would impart an "oceanic facies", i.e. appearance to faunas. Both *Zosterops* and *Nectarinia*, and pigeons generally, show Indian Ocean radiations that could also be included in this category (Warren et al. 2003, 2006). Moreau and Pakenham (1940) considered the high vagility of these taxa to reduce their importance, and concluded from the vertebrate fauna that Pemba could not have been isolated much longer than Unguja or Mafia, implying a Pleistocene separation. The geological evidence for a Pliocene separation was however, restated (Stockley 1942, Kent et al. 1971) and the debate remains unresolved (Pickford 2008). Of course, endemism among such vagile taxa could equally be interpreted as indicating stronger, not weaker, isolation, especially from the mainland. Indeed, Moreau and Pakenham (1940) considered dispersal between the mainland and Pemba particularly difficult because of currents passing through the Pemba Channel. However, currents would be predicted to have the same effect when considering dispersal between the mainland and Unguja. The prevailing wind seasonally alternates in direction (Baker and Baker 2002, Pickford 2008). It seems that Moreau and Pakenham (1940) were arguing for a short but strong period of isolation for Pemba. If correct, Pemba's fauna should be a close relative of the mainland's, in which any impoverishment and imbalance results mainly from extinction. A long period of strong isolation would instead result in an unequivocally oceanic fauna in which impoverishment and imbalance results both from extinction and a low rate of immigration due to dispersal limitation. Endemism in either case should be high relative to that of Unguja, which is thought to have experienced a much shorter period of isolation (that may also have been weaker).

Here we discuss results of the first survey of Pemba's terrestrial mollusc fauna ("land-snails", including slugs) in this context. In principle, land-snails are much less vagile than volant animals, yet there is abundant evidence for long-distance dispersal to islands (e.g. Gittenberger 2007). In East Africa, the lowland land-snail fauna is poorlyknown but now documented in a few coastal forest fragments in which endemism to fragments is high (Tattersfield 1998, Lange and Mwinzi 2003, Rowson 2007). These coastal forests form a region of endemism scattered through the Zanzibar-Inhambane vegetation mosaic of White (1983) and support the majority of the region's narrowrange endemics (Burgess et al. 1998, Burgess and Clarke 2000) which include landsnails (Verdcourt 2000, Rowson 2007). Emberton et al. (1997) found that within Tanzania, both diversity and endemism peaked in the northern coastal forests, those nearest Pemba. The forest on Pemba is greatly depleted outside three small government forest reserves (FRs) (Beentje 1990, Burgess et al. 1998, Baker and Baker 2002). Ngezi FR is the best-known of these. Beentje (1990) drew attention to its mixture of plant species with coastal, montane, Asian and Madagascan affinities. Secondary woodland or thicket (especially clove plantations, often abandoned) covers much of the rest of the island and constitutes intervening or ecotonal habitat between FRs. The land-snail fauna of Pemba has scarcely been published upon prior to this study. Voeltzkow (1923: pp.172, 179, 185) recorded 10 species in a general account of Pemba's natural history; Haas (1929) examined Voeltzkow's material, localising some records and adding an 11th species. Verdcourt (1983, 2000, 2006) did not repeat all these records in his abbreviated checklists for East Africa but did add two other species, making 13 in total. This contrasts with at least 58 species now known from Unguja (Rowson 2007). Our aim was to obtain a more accurate land-snail inventory for Pemba and to clarify its affinities to the mainland and other islands.

Materials and methods

To examine how the land-snail fauna reflects the strength and duration of Pemba's isolation we sought evidence of a) impoverishment; b) imbalance in composition; and c) increased endemism, each relative to Unguja and to mainland coastal forests. This required that as many species as possible were documented. We also aimed to clarify

patterns of species presence or absence between habitat types and between FRs, data on which are currently absent for most Pemban taxa and limited for other Tanzanian coastal taxa.

Survey work was carried out in February 2009. Survey sites were selected in each FR and in additional sites covering most of the island (plus Misali I., a small island nature reserve to the west; Fig. 1B, Table 1). The highest point on Pemba is 95m above sea level so all were at roughly equivalent altitude, but were in different vegetation types and on different underlying soil or bedrock (especially at Ngezi FR, which encloses a complex of different forest types [Beentje 1990]). Survey methodology was a combination of direct search and litter sieving adapted from Tattersfield (1996); collecting effort was quantified although it varied across sites. While no survey can guarantee to find all species, these are two of the most important considerations in surveys of this type (Cameron and Pokryszko 2005). Land-snails were identified with reference to collections and the literature and are deposited at the National Museum of Wales, UK (NMW) and National Museums of Tanzania (NMT). As in Rowson (2007), informal morphospecies names ("sp. A" etc.) are avoided, one advantage being more accurate comparison with other studies.

Results

a) Impoverishment and community diversity

4261 mollusc individuals representing forty-seven species were found in total, with only two previously recorded slug species not refound (Table 2). For each habitat type and FR we recorded approximately 16–68 times as many individuals as species, exceeding Cameron and Pokryszko's (2005) suggested sampling minimum of ten times, so we should have a good first estimate of total richness and its variation. Among habitat types, Ngezi FR (sand/soil) (27 species) and Ngezi FR (coral rag) (23 species) were richer than Msitu Mkuu FR (23 species) or Ras Kiuyu FR (14 species). When both habitat types at Ngezi FR were combined, 33 species in total were recorded; four were found only on sand/soil and six only on coral rag. Although our survey cannot accurately compare abundances (Cameron and Pokryszko 2005) the total number of individuals found at Ngezi was much lower on sand/soil (436) than on the coral rag (1138), despite greater collecting effort (Table 1). When aggregated, non-forest sites hosted the greatest number of species (30) but with a mean of only 11.7 species per site, indicating substantial between-site heterogeneity.

All FRs and habitat types contained at least one species not recorded elsewhere on Pemba. Importantly for conservation, 21 species (approximately 45% of the 47 species found) were found only in FRs, including the 10 rarest species (those represented by the fewest individuals) and all the slugs found. For example, *Curvella disparilis* and *Thapsia curvatula* were found only at Ngezi FR (sand/soil); *Microcystina minima*

Table 1. Survey sites (numbered as in Fig. 1B) and grouped into habitat types. Ngezi FR sites are grouped into two habitat types according to underlying geology. "Person-hours" is the total time spent on direct search and "litter" is the approximate volume of litter sieved (litres). Codes in square brackets are original site names and dates of collection.

			Details	Search	effort
No.	Habitat type	Latitude / longitude	Description	Person- hours / litter	Habitat type total
1		-4.939691 / 39.708538	High moist forest on sandy alluvial soil within reserve less than 1km from entrance [N1,7.2.09]	10 / 8	
2	Ngezi FR	-4.935586 / 39.699225	High moist forest on sandy alluvial soil in centre of reserve [N3,8.2.09]	12 / 64	28 / 104
3		-4.919125 / 39.695277	High forest and swamp forest on dark alluvial soil in north of reserve [N6,11.2.09]	3/8	
4		-4.962009 / 39.706607	High moist forest on muddy alluvial soil in stream valley in south of reserve [N7,11.2.09]	3 / 24	
5	Ngezi FR	-4.946789 / 39.678755	Dry forest on dark, sandy soil on coral rag on Tondooni peninsula within reserve [N2,7.2.09]	12 / 24	22/64
6	(coral rag)	-4.959658 / 39.685578	Dry forest and thicket on dark, sandy soil on coral rag on coast of Tondooni peninsula within reserve [N4,8.2.09]	10 / 40	22704
7	Ras Kiuyu FR	-4.907837 / 39.86269	Dry forest on light, not sandy soil on coral rag in degraded high forest in south of reserve [R1,10.2.09]	10 / 32	10 /32
8	Msitu Mkuu FR	-5.000081 / 39.832091	Moist forest on dark, not sandy soil on coral rag in high forest in north of reserve [M1,10.2.09]	12.5 / 32	12.5 / 32
9		-4.967589 / 39.855051	Mango trees on reddish, sandy soil near Kiuyu School [K1,10.2.09]	1.5 / 0	
10		-5.042864 / 39.73521	Clove and fruit tree woodland on reddish, not sandy soil near Wete [117,11/13.2.09]	6 / 16	
11		-5.235746 / 39.606019	Mature bushland on coral rag in interior of Misali Island [MI,14.2.09]	3 / 0	
12	Non-FR sites	-5.276008 / 39.769607	Clove and fruit tree woodland on dark, not sandy soil near Matuleni [MT,15.2.09]	3/8	26.5 / 48
13		-5.313227 / 39.689677	Clove and fruit tree woodland on reddish, not sandy soil near Wambaa [119,13.2.09]	6/0	
14		-5.387244 / 39.765766	Clove and fruit tree woodland on dark, sandy soil near Chwaka [CH,15.2.09]	3 / 16	
15		-5.379286/ 39.691308	Clove and fruit tree woodland on reddish, not sandy soil near Mtondoni [MO,15.2.09]	4 / 8	

was found only at Ras Kiuyu FR; and *Nesopupa minutalis* was found only at Msitu Mkuu FR. Another 21 species (45%) were found in both FRs and non-forest habitat types, including the 10 most abundant species. These include several taxa treated by Verdcourt (2000) as forest specialists (*Tayloria shimbiensis, Opeas delicatum, Subulona ordinaria*) and at least one previously unrecorded from forest habitats (*Assiminea au-*

Table 2. Species recorded from Pemba. An entry in the "Unguja" column indicates the same species occurs on Unguja (page numbers and figures in Rowson, 2007); "nf" in the final column indicates two previously recorded species, both slugs, not found during the present survey

Species									Re	corded f	or site	s					
Species	Note	Figs.	Unguja (pp. / figs.)	Ngezi]	FR (sar	id/soil)	Ngez (coral	i FR rag)	Ras Kiuyu FR	Msitu Mkuu FR			Non-]	R sites	~		Sit N
				-	3	4	Ś	9	7	8	6	10	11	1	3 1	4 1	10
Assimineidae																	
"Assiminea" aurifera Preston, 1912	3	2	429 / 18		+		+	+	+	+	+		+	+			1
Cyclophoridae																	
Cyathopoma azaniense Verdcourt, 1978	4	13-15						+									_
Cyathopoma pembense sp. n.	1	16-27								+		+		+	+		4
Pomatiidae																	
Tropidophora zanguebarica (Petit, 1850)	Ś	3	432/3				+	+	+	+		+	+	+	+	+	1
Veronicellidae																	
Laevicaulis alte (Férussac, 1821)	6		432			+											
Cerastidae																	
Gittenedouardia conulina (von Martens, 1869)	7	29	433/27				+	+						+			4
Rachis punctata (Anton, 1839)			434 / 29								+						_
Rhachistia braunsi (von Martens, 1869)	8	28	434/31					+	+	+			+				u \
Nesopupidae																	
Nesopupa minutalis (Morelet, 1881)			433 / 9							+							
Chondrinidae																	
Gastrocopta klunzingeri (Jickeli, 1873)			433 / 12						+								1
Succineidae																	
Quickia concisa (Morelet, 1848)			447 / 20	+													_
Euconulidae																	
Afroguppya quadrisculpta (Connolly, 1939)		45-47						+						+			(1
Afropunctum seminium (Morelet, 1873)		48–50	445					+	+								
Microcystina minima (H. Adams, 1867)	6	51-53	445 / 14						+								_
Helicarionidae																	
Kaliella barrakporensis (L. Pfeiffer, 1854)		42-44	446	+		+	+	+		+		+		+			

Species										Rec	orded f	or site	s						
Species	Note	Figs.	Unguja (pp. / figs.)	Nge	ci FR (sand/s	oil)	Ngezi (coral	FR rag)	Ras Ciuyu FR	Msitu Mkuu FR			Non	FR sit	es			No. sites
				-	2	3	4	Ś	9	~	×	6	10	11	12	13	14	5	
Ariophantidae																			
Sitala jenynsi (L. Pfeiffer, 1845)	10	36–38	446												+				1
Urocyclidae																			
Pembatoxon insulare van Goethem, 1975	11		447/40	+	+						+								3
Trichotoxon heynemanni Simroth, 1888	11																		nf
Elisolimax roebucki (Simroth, 1910)	11																		nf
"Dendrolimax" vangoethemi sp. n.	2	11-12, 64-75			+				+	+									3
Thapsia curvatula von Martens, 1897	12		446/4	+	+														2
Thapsia insulsa Preston, 1910	13	39-41												+			+		5
Trochonanina mozambicensis (L. Pfeiffer, 1855)	14	6	438/43	+	+		+	+	+		+	+		+	+	+	+	+	12
Ferussaciidae																			
Cecilioides caliipeplum (Connolly, 1923)			435 / 21	+	+				+		+								4
Achatinidae																			
Achatina (Lissachatina) allisa Reeve, 1849	15		438/43	+	+		+	+	+	+			+		+			+	6
Achatina (Lissachatina) fulica hamillei Petit, 1859	16		438/45								+					+			2
SUBULINIDAE																			
Allopeas gracile (Hutton, 1834)	17		436 / 26			+					+	+		+			+		5
Curvella subvirescens (E. A. Smith, 1890)	18	30		+	+														2
Opeas delicatum Taylor, 1877	19		436 / 23–24		+			+	+	+	+	+	+		+	+	+		10
<i>Opeas lamoense</i> Melvill & Ponsonby, 1892	20		436 / 22	+		+					+	+	+		+	+	+		8
Pseudoglessula (Kempioconcha) subolivacea agg. (E. A. Smith, 1890)			436/36	+	+	+	+	+	+	+	+		+	+	+	+		+	13
Pseudopeas igembiense Connolly, 1923		32	435-436 / 17					+	+										5
Striosubulina striatella (Rang, 1831)	21	31																+	-
Subulina intermedia Taylor, 1877			435 / 25	+	+	+	+	+	+		+		+		+	+			10
Subulina octona (Bruguière, 1789)	22		435 / 32			+					+		+		+	+	+	+	4
Subulona ordinaria Preston, 1910	23	4						+	+		+		+						4

-	-									Rec	orded f	or site	s					
Cies	Note	Figs.	Unguja (pp. / figs.)	Ngez	zi FR (sand/s	(lio	Ngezi (coral	FR rag)	Ras Ciuyu I FR	Msitu Mkuu FR			Non-	FR site	SS		N sit
				1	2	3	4	2	6	7	8	6	10	11	12	13	14 1	5
1877)	24	8	439 / 41	+	+		+	+	+				+			+	-	_
latus (Dohrn, 1878)	25	5, 54-56	440/38	+	+	+	+	+	+		+		+		+	+	+	+
nolly, 1923	26	7, 33–35		+	+	+	+				+		+					
ula (Morelet, 1877)			444 / 47-48		+	+		+	+	+	+		+			+		
) aenigmatica (E. A.	27	57-59									+		+		+			(,)
gen, 1975			441-442 / 54						+									_
, 1910)	28	60–61			+			+	+	+	+		+		+	+	+	<u> </u>
1913)			440 / 57						+									_
ston, 1910)	29	62	452	+						+				+	+			7
(0)			441 / 50	+	+		+		+	+								41
lartens, 1892)	30	63			+	+					+				+		+	41
Aartens, 1869)			442 / 58													+		_
1 Bruggen, 2007			442 / 52						+									_
Total species for site	0			16	20	10	10	15	25	14	23	6	16	8	19	14	12 7	7
otal species for FR / habit:	tat ty	pe			27	2		25		14	23				30			
Total species for Pemb	ba									47 (+	2 nf) =	49						

rifera). Both the Pemba-endemic *Cyathopoma pembense* and the Eastern Arc species "*Gulella*" *aenigmatica* occurred only in Msitu Mkuu FR and in non-forest sites. The remaining five species (approximately 10%) were found only in non-forest habitats. These include the only certainly introduced species (*Striosubulina striatella*) as well as two further taxa treated by Verdcourt (2000) as forest specialists (*Thapsia insulsa* and *Gulella sexdentata*).

b) Imbalance

In overall species richness, Pemba's fauna is dominated by the families Streptaxidae (13 species, 27%), Subulinidae (10, 20%), and Urocyclidae (7, 14%). The order remains the same if Achatinidae are included in Subulinidae. Individually, Ras Kiuyu FR has Euconulidae, and non-forest sites have Cerastidae in third place instead of Urocyclidae, but this may be due to the difficulties of sampling slugs. The Maizaniidae are the only conspicuous coastal forest family that appear to be reliably absent from Pemba.

c) Endemism and affinities

The following three species (6% of the total of 49) are known only from Pemba and we consider them endemic: *Cyathopoma pembense, Dendrolimax vangoethemi*, and *Elisolimax roebucki*. There are no endemic genera or subgenera and all three endemics have close relatives both on the mainland and elsewhere in the western Indian Ocean. *D. vangoethemi* probably occurs in the Usambaras (see below) while *E. roebucki* has had doubts raised over its species status (see Rowson 2007 p. 447). The populations of "*Gulella*" radius on Pemba may be considered a separate species (see Notes, 28). Thus the rate of species endemism could be as low as 2% (considering only *C. pembense* endemic) or as high as 8% if (considering *C. pembense*, both slugs, and the Pemba "*G.*" radius to be endemic). Accepting a 6% rate, a total of 36 (73%) of Pemba's species are also found on Unguja. Of these, 33 also occur on the mainland, sometimes in small areas. The remaining two species (4% of Pemba's total) are known only from Pemba and Unguja: *Pembatoxon insulare* and *Gittene-douardia conulina*. There are doubts about the species status of the latter (see Notes, 7).

Descriptions of new taxa

Museum abbreviations are as follows: BMNH: Natural History Museum, London, UK; IRSNB, Royal Belgian Institute of Natural Science, Brussels, Belgium; MNHN, Muséum national d'Histoire naturelle, Paris, France; RMNH, Naturalis, Leiden, the Netherlands; NMT, National Museums of Tanzania, Dar-es-Salaam, Tanzania; NMW, National Museum of Wales, Cardiff, UK; NMSA, Natal Museum, Pietermaritzburg, South Africa; ZMB, Museum für Naturkunde, Berlin, Germany.

1. Cyathopoma pembense Rowson, sp. n.

urn:lsid:zoobank.org:act:9D732594-3393-417A-9F66-F1459AC232BB Figs 16–27

Type material: (all from TANZANIA: Zanzibar: Pemba Island). Holotype (NMW.Z.2009.013.00001): adult shell stored dry; in leaf litter, near Wete (Locality 10 in Fig. 1 and Table 1), 13 February 2009, leg. B. Rowson, B. H. Warren & C. F. Ngereza. Paratypes (NMW.Z.2009.013.00002-00032): 31 adults and juveniles in 80% ethanol; other data as holotype. Paratypes (NMW.Z.2009.013.00033-00077): 45 adults and juveniles stored dry; other data as holotype. Paratypes (NMW.Z.2009.013.00078-00079), 2 adults gold-coated for SEM; other data as holotype. Paratypes (NMT): 2 adults stored dry; collection data as holotype. Paratypes (BMNH.20100582): 1adult & 1 juvenile stored dry; collection data as holotype. Paratypes (MNHN): 1 adult & 1 juvenile stored dry; collection data as holotype. Paratypes (NMSA.L8207/T2591): 2 adults stored dry; collection data as holotype. Paratypes (RMNH): 2 adults stored dry; collection data as holotype. Paratypes (NMW.Z.2009.013.00080-00096): 17 adults and juveniles in 80% ethanol; in leaf litter, Msitu Mkuu FR (Locality 8 in Fig. 1 and Table 1), 10 February 2009, leg. B. Rowson, B. H. Warren, C. F. Ngereza & paid local collectors. Paratypes (NMW.Z.2009.013.00097-00174): 78 adults and juveniles stored dry; other data as previous. Paratype (NMW.Z.2009.013.00175): 1 adult gold-coated for SEM; other data as previous. Paratypes (NMW.Z.2009.013.00175-00176): 2 adults in 80% ethanol; in leaf litter, near Matuleni (Locality 12 in Fig. 1 and Table 1), 15 February 2009, leg. B. Rowson & C. F. Ngereza. Paratypes (NMW.Z.2009.013.00177-00210): 33 adults and juveniles stored dry; other data as previous. Paratype (NMW.Z.2009.013.00211): 1 adult stored dry; in leaf litter, near Wambaa (Locality 13 in Fig. 1 and Table 1), 13 February 2009, leg. B. Rowson, B. H. Warren, C. F. Ngereza & paid local collectors.

Diagnosis: Shell relatively large (to 4.20mm wide) and strongly depressed. When fresh, with spirally-ridged operculum and characteristic periostracum of radial lamellae peripherally extended into long hairs gathered into points, or much shorter hairs gathered into fringes. When denuded, with relatively few spiral keels.

Description of holotype: Adult shell (Figs 16–18) relatively large for the genus in Africa, 2.25mm x 3.95mm including periostracum, strongly depressed, of approximately 4.5 regularly expanding whorls, with wide, perspective umbilicus. Peristome effectively complete, slightly thickened and flaring, especially basally. Aperture and operculum effectively circular. Operculum calcareous, outer surface with multispiral, blade-like raised lamella of approximately 9 revolutions, weakly convex as a result; inner surface smooth. Protoconch smooth, with irregular malleation discernible only at extreme magnification (Fig. 25). Teleoconch periostracum of fine, extremely close (<0.025 mm apart) radial lamellae, running from suture to suture. Lamellae each prolonged into long, flat periostracal extensions ("hairs") extending well beyond the whorl periphery, forming spiral keels (up to four on the body whorl), with less-pronounced periostracal keels continuing into umbilicus. Periostracal hairs (in life and in fresh shells, whether wet or dried) regularly gathered at their tips to form bunches of six or more hairs (Fig. 25).

Further description from paratypes: The periostracum of C. pembense forms a continuum of variation. At one extreme are individuals with hairs gathered together at their tips (as in the holotype). At the other are those in which the periostracal lamellae form instead a rough, raised periostracal fringe where the lamellae appear cemented together (Figs 19–21; 27). These extremes are more frequent than intermediate forms, but such intermediates do occur, in which the bunches of hairs are irregularly missing, probably worn away (see below). The two extreme forms are sympatric at three of the species' four localities – i.e., at the type locality, at Msitu Mkuu FR, and near Matuleni (Localities 10, 8 & 12 respectively in Fig. 1 and Table 1). At the fourth locality near Wambaa (Locality 13 in Fig. 1 and Table 1), only one individual was found, and was of the fringed form. Both forms include both liveand dead-collected individuals, and both adult and juvenile shells. The size ranges overlap, although the fringed form seems to reach a slightly larger maximum (4.00-4.20mm wide with 4.25-4.5 whorls). Other features of the shell (shape, operculum, and protoconch; Fig. 26) are consistent across all individuals. Shells from which the periostracum has been lost were common but always empty, and cannot be assigned to either form. Such denuded shells (Figs 22-24) have relatively few (up to 8) weak spiral keels on the body whorl (including umbilical part), with fine, extremely close incised radial lines between the keels. All nine live-collected individuals of the fringed form were dissected and a penis was detected in six of them. A penis was not detected in any of nine individuals of the hairy form.

Remarks: This species is attributed to Cyclophoridae: Cyathopoma sensu lato following Emberton (2003). All forms of C. pembense differ from the few other East African Cyclophoridae in being larger and more depressed than C. azaniense Verdcourt, 1978 (Figs 13–15), an undescribed *azaniense*-like species from the East Usambaras (Verdcourt 2006; NMW material examined), and the Malawian C. tres van Bruggen, 2008 (van Bruggen 2008). They are also larger than the Central African *C. papillaris* (von Martens, 1892) and have fewer spiral keels (see van Bruggen 1986). The elaborate periostracum appears to be unique among East African taxa but similar features occur in some southeast African and western Indian Ocean island taxa. C. pembense differs from species of the southeast African Chondrocyclus Ancey, 1898 either in the operculum or in periostracal features; C. putealis Connolly, 1939 and C. trifimbriatus Connolly, 1939 have fringes like C. pembense but very different opercula (see van Bruggen 1986; Herbert and Kilburn 2004). C. pembense is more depressed and differs in periostracum from the Seychelles C. blanfordi H. Adams, 1868 (see Gerlach 2006a, b). Photographs of C. pembense were compared with the BMNH types of several Comoros species attributed to "Cyclotopsis" (nevilli Morelet, 1877, filicum Morelet, 1877, and horrida Morelet, 1887). Although worn, none of these were an exact match for C. pembense. Nor does it agree with the descriptions or figures of any other cyclophorid of the Comoros (see Fischer-Piette and Vukadinovic 1974), Madagascar (Emberton 2003, 2004), the Mascarenes (Griffiths and Florens 2006), the Seychelles (Gerlach 2006a, b), nor any Asian species known to us.

The variation shown by this species is striking. One might consider the extreme periostracal forms separate species, albeit indistinguishable when the shells are denuded. However the presence of intermediates suggests that this is not the case. The variation could result from sexual dimorphism (hairy forms female, fringed forms male) which would explain their occurrence in sympatry. However, sexual dimorphism would not explain the existence of intermediate forms. It would also demand that the three fringed individuals without a penis were immature males rather than females, when equally possible is that fringed forms consist of three females and six males while and all nine hairy individuals were immature. Natural wear and corrosion on the periostracum, presumably from hairy to fringed forms, would explain the latter possibility and account for the continuum of variation. It would not, however, easily explain the existence of live animals of both types (in each case both adults and juveniles in sympatry, where presumably the whole population is exposed to similar factors causing wear and corrosion. Possibly both sexual dimorphism and wear on the shells play a part in this unusual pattern. More speculatively, other alternatives include incomplete speciation or hybridisation between two closely related species.

Distribution: Apparently endemic to Pemba island.

Etymology: pembense, from Pemba island, a noun in the generative case.

2. "Dendrolimax" vangoethemi Rowson, sp. n. urn:lsid:zoobank.org:act:40EB98A1-E93A-47FA-B81C-C4F545FFD298 Figs 11–12, 64–75

Type material (all from TANZANIA: Zanzibar: Pemba Island). **Holotype** (NMW.Z.2009.013.00211): slug 30.0 mm long in 80% ethanol, on understorey foliage during day, Ngezi FR (Locality 2 in Fig. 1 and Table 1), 8 February 2009, leg. B. Rowson, B. H. Warren, C. F. Ngereza & local collectors.

Paratype 1 (NMW.Z.2009.013.00212): slug 37.5 mm long in 80% ethanol; other data as holotype. Paratype 2 (NMW.Z.2009.013.00213): slug 31.0 mm long in 80% ethanol; other data as holotype. Paratype 3 (NMW.Z.2009.013.00214): slug 25.0 mm long in 80% ethanol; other data as holotype. Paratype 4 (IRSNB.IG.31599/MT2317): slug 18.0 mm long in 80% ethanol; other data as holotype. Paratype 5 (NMT): slug 19.0 mm long in 80% ethanol, on understorey foliage during day, Ngezi FR (Locality 6 in Fig. 1 and Table 1), 8 February 2009, leg. B. Rowson, B. H. Warren, C. F. Ngereza & local collectors. Paratype 6 (NMW.Z.2009.013.00215): slug 18.5 mm long in 80% ethanol; leaf litter during day, Ras Kiuyu FR (Locality 7 in Fig. 1 and Table 1), leg. B. Rowson, B. H. Warren, C. F. Ngereza & local collectors.

Diagnosis: Medium-sized slug (to at least 55mm in life) with strong keel prolonged into long caudal appendage, with mantle completely covering shell. Pale to colourless, with dorsum covered in pustules. Viscera not extending far into tail, shell mineralised, jaw with no or weak projection. Radula unique in having up to 280 tiny, tricuspid teeth per half-row. Genitalia broadly similar to other *Dendrolimax*.

Description: Note: points of agreement with an unnamed East Usambara species as discussed by Verdcourt and Polhill (1961) (see below) are marked with "*".

External features: Medium-sized slug (extended length to at least 55mm in life, or 37.5mm in 80% ethanol)*. Tail strongly keeled* and hollowed out behind mantle*; keel

prolonged into long straight caudal appendage* above small caudal pore*. Viscera extending little more than half-way into tail*. Body bell-shaped in cross-section when extended, but able to flatten body considerably. Sole not narrow, evenly tripartite*. Peripodial grooves clear, from tail to genital orifice and head. Mantle fully attached posteriorly, free anteriorly, not grooved, subangulate rather than rounded behind, completely covering shell, lacking a dorsal pore or slit*. Pneumostome in posterior third of mantle*. Genital orifice far forward, near right lower tentacle. No head wart or similar structure detected. Integumental tubercles barely detectable on mantle, tail or cephalopodium; instead, whole dorsum rather densely and regularly covered in hemispherical (rather than prickly) pustules*. Dorsum largely colourless and translucent*, with green, grey or pink tinge*, acquiring a green cast when on foliage; keel white; sole colourless. Diffuse, slate-grey pigment on caudal appendage, bordering keel* and/or in obscure blotches or bands on mantle in some specimens*, absent in others; remains on preservation. Pustules conspicuously white*, remaining so on preservation. Ommatophore retractors grey-ochre on preservation.

Jaw and radula: Jaw solid, semi-lunate, lacking median projection (holotype), or projection very weak (paratype). Radula of holotype broad (3.65 mm wide × 2.20 mm long), of 155 rows (over 50 angular rows per mm length). Teeth extremely small* and extremely numerous*, to nearly 450 in each half-row*, with a central tooth. All teeth (including central tooth) tricuspid*, with very little change across the row, perhaps becoming more s-shaped laterally. Ectocones larger (or at least projecting further) than mesocones in all teeth except central tooth. This is unlike any radula figured in van Goethem (1977) where mesocones are always the largest cusps, and the maximum number of teeth per half-row is around 280, and only in radulae over 5.00 mm wide. The radula form may suggest a microphagous, rather than phytophagous diet.

Shell and pallial complex: Shell unguiform, bilaterally symmetrical, to at least 4.10 mm long, infilled, mineralised and white* (i.e. not fragile as in other *Dendrolimax*). Pallial area well vascularised.

Genitalia: Right ommatophore retractor passes between penis and vagina. No atrial diverticulum or stimulator*. Long flagellum present in place of calc sac*. Epiphallus long, stiff, not spiralling around penis*. Penial retractor short, attaching well below flagellum, perhaps obtaining from diaphragm. Penis with basal sheath, contiguous with penis wall apically, internally with longitudinal pilasters and a basal papilla; similar to that of several *Dendrolimax* (see van Goethem 1977). Bursa copulatrix duct arising low on vagina, bound to it by sheath-like circular muscle fibres. Bursa copulatrix long, weakly clavate, reaching upper part of spermoviduct*. Vagina and free oviduct with a clear, thick-walled sheath*. Hermaphroditic duct extremely short*, barely perceptible between spermoviduct and large yellow ovotestis, which lies near rear of mantle. Albumen gland small, hook-shaped*. No spermatophores were recovered from the Pemba material which may not be fully adult.

Remarks: This distinctive species was found only in FRs. At Ngezi, the slugs were found on the underside of large understorey leaves, up to 2m above ground. The body was held flattened with one optic tentacle protruding (Fig. 12). At Ras Kiuyu FR the species was found in litter. It appears to be undescribed although it (or a similar species) may occur in the East Usambara Mts. Beyond that its affinities are less certain.

Van Goethem (1977) thoroughly revised the known urocyclid slugs of Africa and Madagascar and provided keys to internal characters. The Pemba species keys readily out to Dendrolimacini (sole genus Dendrolimax Heynemann, 1868). The only East African record of the mainly Central-West African Dendrolimax is an unnamed and incompletely described species collected by Verdcourt (1960) from Thika Gorge, Kenya, who indicated a swollen lower oviduct not present in the Pemba species. Moreover the Pemba species differs from all other *Dendrolimax* in the radula, body form, and shell, although the genitalia are similar. It shows much clearer similarities to a taxon referred to as "Genus et species nov." by Verdcourt and Polhill (1961, p. 32-33, fig. 42) from Sigi in the East Usambara Mts. (1961). They said, "This mollusc does not belong to the family Urocyclidae judging by [the] radula but to an isolated subfamily of the Helicarionidae near to the Durgellinae". Van Goethem (1977) could not obtain material of this taxon for his revision but considered it a urocyclid. He treated it as "Species E", incertae sedis after Dendrolimacini and Upembellini along with a "Species D" from Grand Comore to which he noted a similarity in the genitalia, but not the radula. Neither Verdcourt and Polhill (1961) nor van Goethem (1977) were certain whether the specimens of "Species E" or "Species D" were adult. This is an important consideration since slugs may change in appearance as they grow. However, neither Verdcourt and Polhill, van Goethem with his experience of growth series of many taxa, nor other slug workers (e.g. Forcart 1967) could attribute these forms to any known species or genus. Verdcourt and Polhill (1961) noted that there was no absolute criterion, e.g. concerning the size of the albumen gland, for recognising adulthood in urocyclid slugs. Since 1977 there has been little further work on the group in East Africa. The Pemba species, which will probably prove to include also the East Usambara species, is here described provisionally in Dendrolimax. Consideration was given to erecting a new genus but owing to a lack of unique features in the genitalia, and the large number of available genus-group names, is avoided until more data are available.

Although this species is fully limacised, there are also similarities in the body form and genitalia to numerous Afrotropical semi-slug genera, among them *Verrucarion* van Mol, 1970 of West and central Africa (van Mol 1970) and *Malagarion* Tillier, 1979 of Madagascar. The resemblance to the latter extends to the radula and white pustules (see Tillier 1979, Emberton 1994). Van Mol (1970) treated all African genera in Urocyclidae: Urocyclinae or Gymnarioninae. Tillier (1979) considered *Malagarion* to belong, with the Mascarene *Colparion* Laidlaw, 1938 in Helicarionidae: Ariophantinae and not Urocyclinae(/idae). He noted that the radula, but not genitalia, of *Malagarion* was similar to the Asian Durgellini (founded on the Burmese semi-slug *Durgella* Blanford, 1863). Verdcourt and Polhill (1961) had also noted a radula similarity between their East Usambara species and the Durgellinae, although this was not discussed by van Goethem (1977). Certainly the radula would be unique in Urocyclinae sensu van Goethem (1977), and resembles *Malagarion* in the size and number of teeth, and the large ectocones (at least on the more marginal teeth of *Malagarion*). The monophyly of these major groups is questionable while the systematics of tropical Limacoidea is still far from resolved (e.g. Tillier 1979, Hausdorf 1998, Schileyko 2002), but it remains possible that this species is related to one of them rather than other *Dendrolimax*.

Distribution: Pemba island; probably also East Usambara Mts.

Etymology: *vangoethemi*, a noun in the generative case, for Dr. J. L. van Goethem of IRSNB, in recognition of his thorough and highly accessible monograph on Afrotropical urocyclid slugs.

Further notes on selected species

3. "Assiminea" aurifera (Preston, 1912)

Fig. 2

Assimania aurifera Preston 1912: 191-192; pl. XXXI, fig. 9

Notes. Living indiduals were abundant in leaf litter across Pemba, including sites many kilometres from the sea or fresh water (Table 2). As the only African terrestrial assimineid, Verdcourt (2000, 2006) considered *A. aurifera* worthy of a new genus in Omphalotropinae, a group diverse on the Mascarenes and elsewhere (Griffiths and Florens 2006). *A. aurifera* is otherwise recorded only from coastal Kenya and Unguja (Verdcourt 2006). However, its shells are very similar to *Assiminia parvula* Morelet, 1877, described from Anjouan, Comoros (Morelet 1877). Also terrestrial, *A. parvula* is widespread in the Indo-Pacific and was recorded from Aldabra by Gerlach and Griffiths (2002). Solem (1959) discussed how terrestrial assimineids could be dispersed naturally by sea although we note that *A. aurifera* is a common fossil in Pleistocene deposits in southern Tanzania (Reuter et al. 2010). Further data are needed to resolve this. Note: Both *A. aurifera* and *A. parvula* were described under misspellings of *Assiminea* Fleming, 1928: *Assiminia* (Morelet 1877) and *Assimania* (Preston 1912). The genus *Eussoia*, to which *A. aurifera* has been referred, now includes only aquatic taxa (Brown 1980).

4. Cyathopoma azaniense Verdcourt, 1978

Figs 13–15

Cyathopoma azaniense Verdcourt 1978: 15-16; fig. 1

Notes. This species is otherwise known only from the vicinity of Shimoni, Kenya (Verdcourt 1978, 1982), the part of the mainland nearest to Pemba (c. 40km).



Figures 2–12. Living animals of selected species from Pemba (not to scale). 2 "Assiminea" aurifera 3 Tropidophora zanguebarica 4 Subulona ordinaria 5 Gonaxis denticulatus 6 Gulella planidens 7 Tayloria shimbiensis 8 Edentulina obesa 9 Trochonanina mozambicensis 10 Pembatoxon insulare 11 "Dendrolimax" vangoethemi (Paratype 2) 12 D. vangoethemi (Paratype 1).

5. Tropidophora zanguebarica (Petit, 1850)

Fig. 3

Cyclostoma zanguebarica Petit de la Saussaye 1850: 53; pl. III, fig. 5

Notes. This group needs revision. Pemba shells are almost identical to those from Jozani Forest, Unguja, including in microsculpture and the range of colour patterns, differing



Figures 13-24. Cyclophoridae from Pemba. 13-15 *Cyathopoma azaniense* 16-18 *C. pembense* sp. n., live-collected holotype 19-21 *C. pembense* sp. n., paratype (live-collected specimen with periostracal fringe) 22-24 *C. pembense*, sp. n., paratype, dead-collected specimen without periostracum.

mainly in a smaller maximum size and (Unguja 14.0 × 12.5 mm; Pemba 12.0 × 11.5 mm). There is slight variation in the strength of the spiral raised ridges, though none of the shells are as smooth as *zanguebarica* Petit, 1850 or *letourneuxi* Bourguignat, 1889 in historical collections (e.g. NMW, MNHN). It is hard to know whether this is infraspecific variation or not. Voeltzkow (1923) recorded "*Lygatella letourneuxi* (Bgl.)" (sic) from Pemba; Haas (1929) recorded it from Chake Chake. As well as *zanguebarica* and *letourneuxi*, Verdcourt (2006) lists two unnamed "species" from "Zanzibar". Either could

correspond to the Pemba taxon; possibly one biological species encompasses all four. One is said to have an affinity to *letourneuxi* and to *T. scabra* (H. Adams, 1867), an extinct Mauritian species that varies in sculpture according to Griffiths and Florens (2006). Intriguingly, these authors describe (p.53) a population of a smooth species of *Tropidophora* being replaced by a rough one over recent decades. Note: Verdcourt treats all East African *Tropidophora* in subgenus *Otopoma* Gray, 1850, but the Asian type species of this belongs in Cyclophoridae not Pomatiidae (=Pomatiasidae) (see Neubert 2003).

6. Laevicaulis alte (Férussac, 1821)

Vaginulus alte Férussac 1821–1822: 14

Notes. Voeltzkow (1923, p. 179) recorded this species from Pemba as "*Vaginula brevis*" *V. brevis* Fischer, 1872 is considered a synonym of the widespread *L. alte* (Forcart 1953, Verdcourt 2006). We tentatively refer two small juveniles from Ngezi FR to this species.

7. Gittenedouardia conulina (von Martens, 1869)

Fig. 29

Buliminus (Pachnodus) von Martens 1869: 153

Notes. The name Gittenedouardia Bank & Menkhorst, 2008 recently replaced Edouardia auctt. non Gude (Bank and Menkhorst 2008). The slender-shelled East African species of Gittenedouardia differ subtly in shell proportions. Two of the Pemba shells are large enough to be adult, at 9.8 × 5.5mm (Fig. 29) and 8.2 × 4.1mm. These were compared with photographs of the lectotypes of G. conulina, G. metula (von Martens, 1895), and G. sordidula (von Martens, 1897) (all in ZMB) and G. tumida (Taylor, 1877) (in BMNH). G. conulina and G. metula appear to be the ends of a shape continuum from slender with less tumid whorls (conulina) to broad with tumid whorls (metula). The Pemba shells, G. sordidula, G. tumida, the other shells in the G. tumida type lot (see Rowson 2007, p.434), and probably G. metuloides (E. A. Smith, 1899) of Malawi and southern Africa, are each somewhere in the middle. A similar species is recorded from Aldabra (as Edouardia cf. tumida in Gerlach 2006b) and the Comoros Bulimus badiolus Morelet, 1881 also appears to belong to this group. This merits more detailed analysis but for now we associate the Pemba species with G. conulina. This happens to be both the only species previously recorded from Pemba and the oldest available name. It was noted on Pemba by Voeltzkow (1923) and Haas (1929) as Conulinus conulinus (von Martens)

Rowson (2007, p.433–434, 454–455) considered *G. conulina* one of the few taxa recorded from both Unguja and Pemba, but not the mainland. Given the taxo-

nomic problem this is of little biogeographic significance. Regrettably the confusion in this group may have been added to by contradictorily illustrating *G. conulina* with a specimen from the mainland (Kilifi, Kenya) (Rowson 2007: Fig. 27). This bleached specimen's identification (by the late T. Pain) as "*Cerastus conulinus* (Mts.)" was taken at face value, but it is too large (16.1mm) to be either *G. conulina* or *G. sordidula*. The name *sordidula* was introduced by von Martens (1897) to replace the homonym *conulinus* von Martens, 1878 (not von Martens, 1869) after having used the name *conulinus* for what he later considered two separate species in the same genus. As stated above, we consider the lectotypes of these two species, *conulina* and *sordidula* to be very similar, but the drawings in von Martens (1897) rather emphasise the differences which may have helped to mislead Pain. However, whichever species the Kilifi specimen represents, it is not one recorded from Unguja or Pemba.

8. Rhachistia braunsi (von Martens, 1869)

Fig. 28

Buliminus (Rhachis) von Martens 1869: 150

Notes. All Pemba material appears to be conspecific. Across sites, shells reach only c14mm when adult, and are very thin, not glossy, with faint and irregular spiral and radial striae. One or more brown spiral bands per whorl are present on a weak yellow background, some individuals having a few additional weak brown spots. In several individuals the apex is dark. Multi-banded forms match what Verdcourt (1961, 2006) calls *Rhachidina braunsi* var. *quadricingulata* (E. A. Smith, 1890), described from lowland Tanzania. All the Pemba material is here referred to *braunsi*, whose varieties we consider only colour forms. Voeltzkow's (1923) record of "*Rachis brauensis* Mart." (sic) probably refers to *R. braunsi* from Fundu I. (Haas, 1929). The genus is here given as *Rhachistia* rather than *Rhachidina* (see Solem 1959, Mordan 1992, Herbert and Kilburn 2004).

There is a hypothesis that *Bulimus histrio* L. Pfeiffer, 1854, described from the New Hebrides, is a synonym of *R. braunsi*. Solem (1964) cited Verdcourt (1961) as confirming a suggestion in Solem (1959) that *braunsi* and *histrio* were synonyms, thus proving that *histrio* was an early anthropogenic introduction from East Africa. Though not quite correct (the name *braunsi* did not appear in Solem 1959) this was followed by other workers in the region (e.g. Starmühlner 1970) and there is now a consensus that the Australasian populations originated in East Africa (e.g. Stanisic 1998, Herbert and Kilburn 2004). However, Verdcourt (1961, 1983, 2006) remained ambiguous about placing the two in synonymy and objected that the colour pattern in Solem's (1959) black and white picture of a type of *R. histrio* had not yet been noticed among East African species. He also noted the existence of other, earlier names. Our material is available should anyone be in a position to resolve this debate.



Figures 25–32. Cyclophoridae and Stylommatophora from Pemba. 25 *Cyathopoma pembense* sp. n., paratype, periostracal hairs and protoconch of 26 *C. pembense* sp. n., protoconch of paratype with periostracal fringe 27 *C. pembense* sp. n., paratype, periostracal fringe 28 *Rhachistia braunsi* 29 *Gittenedouardia conulina* 30 *Curvella subvirescens* 31 *Striosubulina striatella* 32 *Pseudopeas igembiense*.

9. Microcystina minima (H. Adams, 1867)

Figs 51-53

Macrochlamys minima Adams 1867: 303; pl. 19, fig. 2

= "Afroguppya rumrutiensis (Preston, 1911)" sensu Rowson 2007 (p. 445, fig. 14)

= Dupontia sp. in Gerlach and Griffiths 2002

Notes. The Pemba material matches that from Unguja figured by Rowson (2007) under the name *A. rumrutiensis*, and the "*Dupontia* sp." found on Aldabra (Gerlach and Griffiths 2002). Although these three populations appear conspecific, the species is not the mainly East African *A. rumrutiensis*. The West African *A. solemi* de Winter & van Bruggen, 1992 resembles it closely in size and shape, but has obscure spiral sculpture (de Winter and van Bruggen 1992) whereas these are finely granular. Igor Muratov (pers. comm.; Muratov in press) refers similar material from north-east Mozambique to *M. minima*, a common species on the Mascarenes (see Griffiths and Florens 2006).

10. *Sitala jenynsi* (L. Pfeiffer, 1845) Figs 36–38

Helix jenynsi Pfeiffer 1845: 131

Notes. Although Voeltzkow (1923) did not mention this species Haas (1929) recorded it from Fundu I. as *Trochonanina (Martensia) jenynsi* (Pfeiffer) based on Voeltzkow's material.

11. Pembatoxon insulare van Goethem, 1975

Fig. 10

Pembatoxon insulare van Goethem 1975: 207-216

Notes. This species was found only in FRs (Table 2) in leaf litter and rotting wood. The only spermatophore obtained was partially digested but the slug recalls *P. insulare* in all other respects including the radula. Van Goethem (1975) noted the holotype (BMNH) was collected in 1901 from "Pemba Island, E. Africa" by C. Crossland but gave no further locality.

Two additional urocyclid slugs were recorded from Pemba by Voeltzkow (1923, p.173) who noted their appearance after heavy rain. These were the only previously recorded speices not relocated in our survey. The difficulties in inventorying slug faunas are well-known (Cameron and Pokryszko 2005) so this does not necessarily imply their absence. They are *Elisolimax roebucki* (Simroth, 1910) (as *Urocyclus roebucki*) and *Trichotoxon heynemanni* Simroth, 1888 (as *T. voeltzkowi* Simroth, 1910).

12. Thapsia curvatula von Martens, 1897

Thapsia curvatula von Martens 1897: 41; pl. III, fig. 12

Notes. Found only in high forest at Ngezi (Table 2), supporting Verdcourt's (2000) suggestion that it is a forest specialist.

13. Thapsia insulsa Preston, 1910

Figs 39-41

Thapsia insulsa Preston 1910: 531; pl. VIII, fig. 14

Notes. The small size, tight coiling, and large umbilicus of this species are distinctive even within this difficult genus (Preston 1910). Verdcourt (2006) records *T. insulsa*



Figures 33–56. Stylommatophora from Pemba (except where noted). 33–35 *Tayloria shimbiense* 36–38 *Sitala jenynsi* (Marafa, near Malindi, Kenya) 39–41 *Thapsia insulsa* 42–44 *Kaliella barrakporensis* (Mt. Elgon NP, Kenya) 45–47 *Afroguppya quadrisculpta* (Udzungwa Mountains NP, Tanzania) 48–50 *Afropunctum seminium* 51–53 *Microcystina minima* 54–56 *Gonaxis denticulatus*.

from the Shimba Hills (the type locality) and Mrima Hill, and NMW has specimens from Gazi (all localities in coastal Kenya). We found only in non-forest sites (Table 2).

14. *Trochonanina mozambicensis* (L. Pfeiffer, 1855) Fig. 9

Helix mozambicensis Pfeiffer 1855: 91-92; pl. XXXI, fig. 9

Notes. Occurs in all habitat types (Table 2). Voeltzkow (1923) recorded it from Pemba as "*Trochonanina mossambicensis* Pts." and "*Trahonemia mossabicensis*" (sic), noting numerous individuals. Haas (1929) recorded it as *Trochonanina mossambicensis* (Pfeiffer) from Chake Chake and Fundu I.

15. Achatina (Lissachatina) allisa Reeve, 1849

Achatina allisa Reeve 1848-1850: pl. V, fig. 16

Notes. Widespread on Pemba (Table 2). Some individuals from high forest at Ngezi are small (to 61mm high) with a very irregularly thickened outer lip, suggesting adulthood.

16. Achatina (Lissachatina) fulica ?subsp. hamillei Petit, 1859

Achatina fulica Petit de la Saussaye 1859: 384-5; pl. XIII, fig. 3

Notes. Large shells agreeing with *hamillei* (see Rowson 2007) were the only *Achatina* found at Msitu Mkuu but apparently did not occur in other FRs (Table 2). Voeltzkow (1923) noted that "*Achatina fulica* (Fer.)" (sic) was widespread on Pemba; Haas (1929) gave a record from Fundu I.

17. Allopeas gracile (Hutton, 1834)

Bulimus gracile Hutton 1834: 84, 93

Notes. Pemba specimens reach a large size $(13.5 \times 3.4 \text{ mm}, \text{ occasionally } 15.0 \times 3.5 \text{ mm}, \text{ up to 8 whorls})$, always being relatively narrower than *Opeas lamoense*. The maximum size is substantially greater than given by most authors (e.g. Gerlach 2006b: 9.4 mm; Griffiths and Florens 2006: 13.5 mm). Said to be restricted to "waste places" by Verdcourt (2000) this species nonetheless occurs in some forest habitats (Table 2). *A. gracile* is widespread in the tropics but its native range

is uncertain. Voeltzkow (1923) noted that "*Opea gracile johanninum* (Mor.)" (sic) was numerous in rotting logs on Pemba; Haas (1929) gives the record from Chake Chake. Fischer-Piette and Vukadinovic (1974) consider *Bulimus johanninus* Morelet, 1877, described from the Comoros, a synonym of *A. gracile*. Recent authors (e.g. Neubert 1998, Gerlach 2006b, Griffiths and Florens 2006) rather than Verdcourt (2006) and Rowson (2007) are followed here in treating this species in *Allopeas* as distinct from *Lamellaxis*.

18. Curvella subvirescens (E. A. Smith, 1890)

Fig. 30

Bulimus (Hapalus) subvirescens Smith 1890: 156; pl. 5, fig. 12

Notes. This species reaches 12.0×5.0 mm and is relatively broader than Pemba's other subulinids. It was found only in high moist forest at Ngezi (Table 2). It keys out to *C. subvirescens* using Verdcourt's (2002) key and resembles the types at BMNH. Verdcourt (2006) records *C. subvirescens* from the Nguru Mts. and Uluguru Mts. and notes its similarity to the Tanzanian *C. sinulabris* (von Martens, 1878) and Kenyan *C. pertranslucens* Preston, 1910, the latter described from the Shimba Hills (Preston 1910).

19. Opeas delicatum Taylor, 1877

Opeas delicatum Taylor 1877b: 281-282; pl. III, fig. 3

Notes. Pemba specimens reach 7.0×2.5 mm, being much smaller and narrower than *O. lamoense*. Griffiths and Florens (2006) figure specimens referred to *Allopeas clavulinum* (Poitiez & Michaud, 1838) and *A. mauritianum* (L. Pfeiffer, 1847) that they suggest were introduced to the Mascarenes from East Africa. Both resemble some specimens of *O. delicatum*. Verdcourt was familiar with *A. clavulinum* in botanic gardens in the UK, which in turn have been said to come from East Africa (Kerney and Cameron 1979) yet never included *A. clavulinum* in his East African lists (Verdcourt 1983, 2000, 2006). This should be further investigated.

20. Opeas lamoense Melvill & Ponsonby, 1892

Buliminus lamoense Melvill and Ponsonby 1892: 90; pl. V, fig. 12

Notes. Pemba specimens reach 11.0×4.0 mm, being relatively much broader and with a relatively larger body whorl than *A. gracile*. At one high forest site (Ngezi N2) some individuals have much stronger ribs, although the shell shape is similar.

21. Striosubulina striatella (Rang, 1831)

Fig. 31

Helix (Cochlicope) Rang 1831: 34-35; pl. III, fig. 7

Notes. Found only at Mtondoni (Table 2). The genital anatomy conforms exactly with that of *S. striatella* as figured by Schileyko (1999), though the bursa copulatrix is less voluminous. A tropical West African species, *S. striatella* has been widely introduced including to the Mascarenes (Griffiths and Florens 2006). This appears to be the first record from East Africa.

22. Subulina octona (Bruguière, 1789)

Bulimus octona Bruguière 1789: 325

Notes. The geographical origin of this species is unknown. Gerlach (2006b) notes it has been found as a subfossil on Aldabra but Griffiths and Florens (2006) suggest it is originally Neotropical. Verdcourt (in litt. 2006) noted that he knew of no material of *S. octona* from East Africa so it is possible the species is spreading. The date of authorship follows Bank and Menkhorst (2008).

23. Subulona ordinaria (Preston, 1910)

Fig. 4

Homorus ordinaria Preston 1910: 534; pl. IX, fig. 25

Notes. Verdcourt (2000) treated this as a forest species but it also occurs in other habitats on Pemba (Table 2). It is known from the Shimba Hills (Preston 1910; type locality) and from the Sigi Valley in the East Usambara Mts. (Verdcourt 2006).

24. Edentulina obesa (Taylor, 1877)

Fig. 8

Buliminus obesa Taylor 1877a: 255; pl. II, fig. 3

Notes. This species was previously recorded from Pemba by Verdcourt (1983, 2006).

25. Gonaxis (Gonaxis) denticulatus (Dohrn, 1878)

Figs 5, 54-56

Streptaxis denticulatus Dohrn 1878: 152

Notes. This species is widespread and abundant on Pemba (Table 2). The specimens are assigned to the widespread *G. denticulatus* pro tem. rather than the more restricted *G. gibbonsi* Taylor, 1877. A revision of the East African taxa attributed to "*Gonaxis*" is currently under way (Rowson in prep.).

26. Tayloria shimbiensis Connolly, 1922

Figs 7, 33–35

Tayloria shimbiensis Connolly 1922: 487

Notes. This species has not previously been recorded beyond the type locality (Shimba Hills) (Verdcourt 2006). According to Verdcourt (1958), its shell differs from that of the other known lowland species, *T. helicoides* (C. R. Böttger, 1913) only in relative spire height and strength of sculpture. The latter is known only from Kipatimu, Kilwa District, south of the Rufiji Delta.

27. *"Gulella" (Aenigmigulella) aenigmatica* (E. A. Smith, 1890) Figs 57–59

Ennea aenigmatica Smith 1890: 164; pl. 6, fig. 11

Notes. Pemba material matches Usambara material (NMW) of this Eastern Arc species very well. No species of *Aenigmigulella* has previously been reported from the coastal region (Verdcourt 2000, 2006) and it is unknown from Unguja. If native to Pemba it would thus suggest a special Eastern Arc affinity. However we note it was not found in forest reserves apart from a juvenile at Msitu Mkuu FR (Table 2). On Pemba, juvenile shells have complex dentition at a variety of stages. The living animal is cream-coloured. Recent systematic work (Rowson, 2010) indicates "*G.*" *aenigmatica* does not belong in the genus *Gulella* L. Pfeiffer and a genus-level revision is in progress.



Figures 57–63. Streptaxidae from Pemba. 57–58 "Gulella" (Aenigmigulella) aenigmatica 59 "G." (A.) aenigmatica, juvenile 60 "G." radius 61 "G." radius, juvenile 62 G. gwendolinae (resembling var. scissidens) 63 G. planidens.

28. *"Gulella" radius* (Preston, 1910) Figs 60–61

Ennea radius Preston 1910: 529; pl. VII, fig. 8

Notes. As noted by Verdcourt (1985) there is either substantial shell variation in the species *Ennea radius* Preston, 1910, or it comprises a complex of related taxa. This cannot be resolved without a thorough revision. Preston's (1910) type from the Shimba Hills is said to be 3.25mm high and is strongly acuminate. Verdcourt (1985) figured a specimen from Diani Beach, Kenya which is much less acuminate but measures 4.23mm (calculated from his drawing). At up to 5.45mm, Pemba specimens are larger still, but resemble the type in being strongly acuminate and with less tumid whorls than the Diani Beach material. Specimens referred to *G. radius* from Unguja (Rowson 2007) are small (to 3.0mm) and not strongly acuminate. The peristomal teeth are in the same basic pattern in each of these populations, but vary in their size and complexity, none being quite as different as some of the other nominal species (discussed in Rowson and Lange 2007). What does appear relatively constant is the size, shape, and sculpture of Pemba specimens, which occur throughout the island (Table 2). They may form an island taxon worthy of subspecies or species status, which is given consid-

eration in the discussions on endemism in the present paper. Recent systematic work (Rowson 2010) indicates "G." radius does not belong in the genus Gulella L. Pfeiffer and a genus-level revision is in progress.

29. Gulella gwendolinae (Preston, 1910)

Fig. 62

Ennea gwendolinae Preston 1910: 527; pl. VII, fig. 3

Notes. Six of the seven adults from Misali Island have an additional palatal tooth, recalling var. *scissidens* Connolly, 1922, described from Dar-es-Salaam. The additional tooth is not present on either of the two adults from Ras Kiuyu. This is a very widespread species and several such forms have been named. Neubert (1998) points out a discrepancy between Paladilhe's (1872) description and figure of *Ennea isseli* Paladilhe, 1872 from Yemen, and figures an additional specimen which strongly resembles *G. gwendolinae*. This raises the possibility that *E. isseli* is a senior synonym of *G. gwendolinae* and, if so, also the question of whether it is truly native to Arabia. A direct comparison of types is advised.

30. Gulella planidens (von Martens, 1892)

Fig. 63

Ennea planidens von Martens 1892: 179

Notes. This species, widespread in East and South-east Africa (van Bruggen and van Goethem 1997) was much more frequent in non-forest habitat types than in forest (Table 2). Voeltzkow (1923) recorded "*Gulilla laevigata* (Dohn)" (sic); Haas (1929) recorded *G. laevigata* (Dohrn) from Chake Chake. The record probably actually refers to *G. planidens* (see van Bruggen and van Goethem 1997).

Discussion

a) Impoverishment and community diversity

Though the number of species varies between FRs and habitat types, the total number of species for Pemba is not low. Richness values for each of the FRs are within the range for those of moderately rich to rich coastal forests in the region (18–50 species) (Emberton et al. 1997, Tattersfield 1998, Lange and Mwinzi 2003, Rowson 2007). Emberton et al. (1997) found northern Tanzanian coastal forests were richer than southern ones. They found Amboni caves (due west of Pemba on the mainland,



Figures 64–75. "*Dendrolimax*" vangoethemi sp. n. from Pemba. 64–68: Radula of holotype: 64 marginal teeth 65 lateral teeth 66 central teeth including median tooth (M) 67 whole radular row 68 whole radula. 69–71 genitalia of Paratype 2: 69 genitalia 70 free oviduct and sheath 71 penis and sheath; 72–73 shell of holotype: 74 jaw of holotype 75 tail of Paratype 2 in 80% ethanol. Abbreviations: **ag** albumen gland **at** atrium **bc** bursa copulatrix **ca** caudal appendage **ep** epiphallus **ff** foot fringe **fl** flagellum **fo** free oviduct **ke** keel **M** median radular tooth **ot** ovotestis **pe** penis **pr** penial retractor **sh** sheath **vd** vas deferens **vg** vagina.

at less than 100m elevation) to be the richest site of all with 50 species; Tattersfield (1998) also found it to be the richest but recorded only 29 species. The difference probably partly reflects taxonomic discrepancies in the morphospecies approach. At least 29 species are recorded from Jozani Forest on Unguja (Rowson 2007). When both habitat types at Ngezi FR are combined, the total of 33 species makes it one of the richest of all East African coastal forests, exceeding some with a much larger area including Arabuko-Sokoke Forest in Kenya (25 species; Lange and Mwinzi 2003). When the two habitat types at Ngezi FR are considered individually, they are still relatively rich (27 on sand/soil and 25 on coral rag). Thus each of Pemba's FRs supports a fauna of typical richness for northern coastal Tanzania, while Ngezi is especially rich, meaning none are strongly impoverished relative to forests on the mainland. The total recorded fauna of Pemba itself (49 species) is approximately 15% lower

than that for Unguja (58 species) so appears slightly impoverished overall. However, the latter is a slightly larger island and has received far more collecting attention historically. There is no data from Mafia or from a comparably-sized area of lowland habitat from the mainland apart from Arabuko-Sokoke. There, in a total area of 372 km² consisting of several forest habitat types, 1263 individuals (comparable to the total in our survey) were recorded representing just 25 species (Lange and Mwinzi 2003). Thus we conclude that there is no good evidence that Pemba's land-snail fauna is impoverished.

The contrast in species composition between habitat types at Ngezi FR shows the importance of its diversity of habitats (cf. Beentje 1990). The apparent difference in abundance could be explained by the apparently rapidly draining sandy soil reducing available moisture. Alternatively, soil pH (the only factor found to significantly influence land-snail abundance at Arabuko-Sokoke; Lange 2003) could explain the difference. Species richness is low to intermediate outside FRs, indicating non-forest habitats are suitable for at least part of Pemba's land-snail fauna. This includes several species previously characterised as forest specialists by Verdcourt (2000). Unless this simply reflects a lack of information available to Verdcourt (2000), it suggests that Pemba's woody cultivation and moist climate might permit locally-adapted species to persist in a broader range of habitats than their mainland counterparts. A similar observation has been made for Pemba's endemic birds (Catry et al. 2000).

b) Imbalance

Across land-snail families, species were recorded in the same rank order and approximately the same proportions as on Unguja (Rowson 2007) and the coastal region as a whole (Verdcourt 2000). Emberton et al. (1997) gave figures on these proportions for coastal forests; though not strictly comparable because of taxonomic discrepancies, the rank order of the three main families is the same and the proportions similar for most forests. The major part of the fauna thus provides no strong evidence of imbalance compared with neighbouring continental areas.

The absence of Maizaniidae (i.e. *Maizania*) is worthy of comment. We are unlikely to have overlooked the durable, conspicuous and often abundant shells of this group at our sites. It occurs in suitable habitat throughout East Africa including Arabuko-Sokoke and the Usambaras; one species is known from the coast, and one from Unguja (Lange and Mwinzi 2003, Verdcourt 2006, Rowson 2007). However, *Maizania* is absent from the Pliocene-Pleistocene central highlands of Kenya, a montane forest area of apparently suitable habitat (Verdcourt 1984). Its absence from Pemba could suggest Pemba, unlike Unguja, was isolated before *Maizania* could reach it. Alternatively, *Maizania* may have reached Unguja only after it became an island, in which case its failure to reach Pemba would have to be explained by stronger isolation by the Pemba Channel. Beyond suggesting the two islands were isolated independently,

which clashes with the distribution of *Pembatoxon insulare* (see below), the absence of *Maizania* does not resolve the question of oceanicity.

c) Endemism and affinities

The rate of endemism (4-8% of species) is very similar to that on Unguja. There, three species (5% of a total of 58) were considered endemic by Rowson (2007) who considered this a rate comparable to coastal forests on the mainland. There are doubts over the species status of several of these taxa, so interpretations of 8% for Pemba vs. 0% for Unguja, or 2% for Pemba vs. 5% for Unguja are possible but, in our opinion, not justified if the margins of uncertainty in taxonomy are taken into account. There are no endemic genera or subgenera. Species with clear affinities to more distant faunas, e.g. Madagascar, the Mascarenes or Asia have been noted in other groups on Pemba (e.g. Moreau and Pakenham 1940, Beentje 1990, Dijsktra et al. 2007). The endemic species have apparently close relatives on the Comoros, but also on the mainland, so are not unusual among Pemban taxa or indeed the rest of the Tanzanian coastal fauna (Rowson 2007, and unpubl. obs.). The relict Gonospira expatriata Preston, 1910, which has apparent Mascarene affinities, and was (or is) extant in coastal Kenya (Verdcourt 2000) has not been found on Pemba. Unless the island once supported additional endemics that are now extinct, these patterns argue against a long history of isolation, especially one many times longer than that of Unguja. We admit to finding this surprising in the light of other taxa reported from Pemba and the debate over its isolation. It is possible that Pemba's land-snail fauna has suffered disproportionately from post-isolation extinction e.g. by drought, fire, tsunami, or sea-water inundation, explaining the lack of endemics. Recent work indicating a rapid uplift by 80-110m in parts of coastal Tanzania over the last 44,000 years (Reuter et al. 2010) raises the question of whether part or all of Pemba subsided below sea level before this time and was subsequently reuplifted. However, sea level itself was 50m or more lower than present during this period (see Reuter et al., 2010) and inundation would not explain the survival of endemics in other taxonomic groups.

Conversely, 92–98% of Pemba's land-snail species occur elsewhere. To date about 8 of these (16%) are known only from small areas of adjacent Tanzania or Kenya (*Cy-athopoma azaniense, "Gulella" aenigmatica, Tayloria shimbiensis* etc.) and could comprise a vicariant fauna whose ranges were split only by the Pemba Channel graben. Alternatively, these and the remainder that occur more widely (*Gulella planidens, Streptostele acicula*, etc.) could have arrived by post-isolation dispersal, with species occurring nearby most likely to arrive soonest. Successful dispersal to Pemba argues against an especially strong isolation, since gene exchange with the mainland would remain possible. This contrasts with Pemba's volant species for which winds are thought to have strongly limited westward passage from the mainland (Moreau and Pakenham 1940; Baker and Baker 2002). Although accidental introduction by man has played a largely unknown but probably greater part in the land-snail fauna, subfossils on Aldabra (e.g. Gerlach and Griffiths 2002) indicate natural, overseas dispersal by land-snails

in the region. Rivers outflowing eastwards from the mainland (e.g. the Pangani, Wami and Ruvu) could aid the dispersal of rafting taxa such as land-snails to the islands, even against seasonally prevailing currents. This could explain the discrepancy with the endemism in volant taxa.

At least 70% of Pemba's fauna is shared with Unguja. Two taxa (4% of Pemba's fauna, or 3.5% of Unguja's) appear to be restricted to both islands so could signal a recent connection or successful post-isolation dispersal between them. However, the slug *Pembatoxon insulare* is the only well-characterised species of the two, and is identifiable with certainty only from spermatophores, so may yet have been overlooked on the mainland. This seems inconclusive evidence on which to propose a vicariant relationship between Unguja and Pemba, while at least one absence (*Maizania*) suggests they were isolated independently.

Conclusion

Pemba's fauna as revealed by our survey shows no unequivocal evidence of impoverishment, imbalance, or a high rate of endemism so appears little or no more oceanic than Unguja's. Two land-snail species distributions that might result from older vicariance give conflicting signals: that of Maizania suggests the islands were isolated independently, while that of *Pembatoxon insulare* suggests they were not. Therefore, although various interpretations are possible, the current consensus from geological data that Pemba has been isolated for much longer than Unguja is not reflected in the snail fauna of the two islands. This phenomenon seems most likely explained by the Pemba channel being a weak barrier to land-snail dispersal, which might explain the discrepancy with the endemism rate of volant taxa. Nonetheless, in the light of our results a critical re-examination of the geological data on the formation of the Pemba channel – especially the timing of the graben faulting and the rate of land subsidence - would be worthwhile. Despite Pemba's snail fauna lacking the signature of a long period of isolation, this island, and in particular its three FRs, does support endemic land-snail species, and several otherwise found only in small areas of the mainland. This makes its fauna of global conservation importance. Evidence of at least one introduction not yet noted elsewhere in East Africa gives some cause for concern. Moreover, the discovery of undescribed taxa suggests much remains to be learnt about land-snails in this region of endemism.

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RESEARCH ARTICLE



Five additions to the list of Sepsidae (Diptera) for Vietnam: Perochaeta cuirassa sp. n., Perochaeta lobo sp. n., Sepsis spura sp. n., Sepsis sepsi Ozerov, 2003 and Sepsis monostigma Thompson, 1869

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Abstract

A recent collecting trip to Vietnam yielded three new species and two new records of Sepsidae (Diptera) for the country. Here we describe two new species in the species-poor genus *Perochaeta (Perochaeta cuirassa* **sp. n.** and *P. lobo* **sp. n.**) and one to the largest sepsid genus *Sepsis (Sepsis spura* **sp. n.**) which is also found in Sumatra and Sulawesi. Two additional *Sepsis* species are new records for Vietnam (*Sepsis sepsi* Ozerov, 2003; *Sepsis monostigma* Thompson, 1869). We conclude with a discussion of the distribution of *Perochaeta* and the three *Sepsis* species.

Keywords

Sepsidae, Perochaeta, Sepsis, new species, new records, Vietnam

Introduction

The Sepsidae are a moderately large, cosmopolitan family of saprophagous flies, with over 300 extant species recorded from all zoogeographic regions (Ozerov 2005). Most species are attracted to dung, carrion, and other malodorous, decaying organic substrates (Pont and Meier 2002); i.e., by using different substrates in different microhabitats, the sepsid fauna from a specific locality can be quickly explored. Separating sepsids from the remaining saprophagous insects is also relatively straightforward because most sepsids can be easily recognized based on the constriction of the first two abdominal segments which gives the flies a wasp- or ant-like habitus.

Here we update an existing species list for Vietnam by adding five species: three are new to science while two others are new records. The relatively large number of additions is due to the fact that the Vietnamese sepsid fauna remains poorly studied (e.g. Ozerov 1993, Iwasa and Thinh 2008). The current species list comprises 21 species in six genera and is based on the sepsid world catalogue (Ozerov 2005) and subsequent taxonomic research by Iwasa and Thinh (2008). We complement this list by adding five species that were collected during a brief collecting trip in July 2010: *Perochaeta cuirassa* sp. n., *P. lobo* sp. n., *Sepsis spura* sp. n., *S. sepsi* and *S. monostigma*.

Materials and methods

All five species were collected between 11–16 July 2010 from Northern Vietnam (Ba Vi National Park and Sa Pa Valley). Cow dung was placed in various habitats for at least a few hours to attract sepsids, which were then caught by sweep-netting. Additional material for *Sepsis sepsi* and *S. spura* were also collected previously in Indonesia (Sulawesi and Sumatra) in 2007 and 2009.

Specimens were photographed using a Leica Z16 APO-A stereomicroscope fitted with a DFC425 digital microscope camera, and then digitally traced to illustrations using a Wacom© PTZ 630 tablet. We also amplified and sequenced a 544-bp fragment of cytochrome oxidase c subunit I (COI) within the DNA barcoding region for the two new *Perochaeta* species based on the methods described in Tan et al. (2010). All type specimens and additional material are vouchered in 95% ethanol and kept in the Cryogenic Collection of the Raffles Museum of Biodiversity and Research (RMBR), National University of Singapore, Singapore. We adopt the taxonomic terminology as described by Merz and Haenni (2000) for adult morphology (excluding terminalia) and Sinclair (2000) for male genitalia.

Taxonomy

Describing new species in genera that have not been revised recently requires extra care and justification, because the risk of creating new synonyms based on overlooked or misinterpreted species in the literature is high. Fortunately, this is not the case for *Pero*- *chaeta*, which has only three described species (see Ozerov 2005 and Ang et al. 2008) and no synonyms. In addition, the descriptions and illustrations for the described species are of good quality. Furthermore, molecular data are consistent with distinct species: the two new *Perochaeta* species are separated by 3.3% for the barcoding gene COI (uncorrected pairwise distances); while the distances of either to *Perochaeta dikowi* are 11.4% and 11.8% (see Table 1 for variable base pairs).

Describing a new Oriental Sepsis species in the absence of a generic revision is more problematic given that the genus is the largest in Sepsidae (ca. 80 described, valid species). Of the 23 Sepsis recorded in the Oriental region (Ozerov 2005), the Sepsis species described here closely resembles the widespread S. nitens Wiedemann, 1824 which has two synonyms (S. brevicosta Brunetti, 1910 and S. tuberculata Duda, 1926). Duda's (1926) description and fore leg illustration of S. tuberculata are sufficiently detailed to confirm that it is indeed a synonym of S. nitens. However, Brunetti's (1910) rather vague description of S. brevicosta based on one male from Calcutta and a few females from localities in other Indian localities (Calcutta, Shencotta, Tinpahar and Pusa), is more difficult to interpret. He describes S. brevicosta's fore femur as having "a small bump [on the ventromedial region] with three or four strong short spines". This description of a "bump" is in agreement with S. nitens (Fig. 21) while the ventromedial protrusion of the new species of Sepsis is shaped more like a spur (Figs 24, 26). In addition, Brunetti's S. brevicosta is known from India, while the new Sepsis species described here is only known from Vietnam (Lào Cai) and Indonesia (Sumatra and Sulawesi).

As argued elsewhere, new species are hypotheses that are dependent on species concepts; it is therefore desirable that authors who describe species are explicit about which species concept was used and whether other species concepts would come to different conclusions (Laamanen et al. 2003, Tan et al. 2008, 2010). Here we apply the Hennigian species concept (Meier and Willmann 2000) and use morphology and DNA sequence data (for *Perochaeta*) to estimate the species boundaries. The two new *Perochaeta* species are sympatric and the new *Sepsis* species is parapatric with *S. nitens*. In both cases we have not seen any intermediate specimens so that there is no evidence for hybridization. This supports our species hypotheses. However, both *Perochaeta* species are so rare that this test is relatively weak. As pointed out by Lim

Table 1. Seventy-two variable base pairs in a 544-bp	COI sequence fragment for three Perochaeta (Posi-
tions according to <i>Drosophila melanogaster</i> COI).	

	0000000000011111111122222222223333333333
Base Pair No.	455666778899223334457800223444556770000112222367888991122244599011245677
	615036251713362384701614251367281094679254578668147061736724325709895779
Perochaeta dikowi	ТСТТАТТАGTNGTATCTCCAAAAAAGAACCAAAATCTTATTATACTTTTACTTATTCTTATCTAAACATTCT
Perochaeta cuirassa	CCACTAAGCATACTATCTTGTTGTGATATTAACGATACTCCTCTTCTGCAGATTAATCCTATAAATTTAATA
Perochaeta lobo	СТАСТАААСАТАТТАТСТТGTTATGATCTTGGTGATACTTTTCTTCCACGGACTAATCCTACAGCTTTAACA

et al. (in press), in such cases descriptions should only be prepared if the species are unusually distinct. This is the case here, whereby the two new species can be clearly distinguished based on both morphology [cf. sternites and hypopygia of *P. cuirassa* (Figs 1–4) and *P. lobo* (Figs 6–9)] and DNA sequence data (Table 1). When the distributional and morphological data are applied to the remaining species concepts in Wheeler and Meier (2000), most support the same species boundaries. The only exception is Mishler and Theriot's (2000) phylogenetic species concept that requires a phylogenetic analysis before species can be delimited. However, such analyses are currently unavailable. We now describe the new *Perochaeta* and *Sepsis* species and state the new records for the two *Sepsis* species.

Perochaeta cuirassa Ang 2010, sp. n.

urn:lsid:zoobank.org:act:76BA1CDF-D467-45D0-9898-305C21045C6D Figs 1–5

Material. *Holotype.* ♂ (RMBR), **Vietnam,** Lào Cai Province, Sa Pa Valley. Baited with cow dung at forest edge next to a small cascade alongside highway, ca. 850m along the road westward of the Thác bạc (Silver Waterfall) tourist attraction [22°23'23.90"N 103°44'50.32"E, elevation 2600m above sea level, ASL]. Collected 16.VII.2010 (Ang Y). *Paratypes.* 2 ♂ (RMBR), collected from same locality and time as holotype.

Etymology. The specific epithet refers to the shape of the main scleral plate for the 4th sternite, which resembles a cuirass or breastplate armor.

Diagnosis. Adult male Perochaeta cuirassa is very similar to Perochaeta lobo and can only be reliably distinguished from the latter based on the 4th sternite [cf. P. cuirassa (Fig. 1) and *P. lobo* (Fig. 6)]: The sternite in *P. cuirassa* lacks distinct lobes on the posterior end of the 4th sternite, while the sternite brush is thick and squat (as opposed to long and thin in *P. lobo*), and the main scleral plate is much broader (long as wide) than in *P. lobo* (twice long as wide). The hypopygium [cf. P. cuirassa (Figs 2–4) and P. lobo (Figs 7–9)] is also distinct, with *P. cuirassa* bearing a large median, decussating protrusion on the dorsal side of the surstylus, while *P. lobo* has a sub-median protrusion on the ventral side of the surstylus. Perochaeta cuirassa is also readily distinguished from all other Perochaeta species based on the morphology of the 4th sternite and hypopygium: The sternites brush of P. cuirassa (Fig. 1) has significantly more bristles (>40 per brush) than either P. hennigi Ozerov, 1992 (Fig. 10) or *P. dikowi* (Fig. 12), both of which have only 5-6 large bristles in addition to a few weaker bristles. Perochaeta cuirassa also has strong bristles lining the distal margin of the sternite, which are not found in *P. dikowi* or *P. hennigi*. The surstylus of *P. cuirassa* (Fig. 2) resembles that of *P. hennigi* (Fig. 11), but can be distinguished by the large median surstylus projection, which is long and curved in *P. cuirassa* but short and broadly triangular in P. hennigi. Both P. dikowi (Fig. 13) and P. orientalis (De Meijere, 1913) (Fig. 14) lack large median projections. *Perochaeta cuirassa* can further be distinguished by the radial-medial cross-vein dividing the discal-medial cell which is in a ratio of 3:1 in P. cuirassa, 2.5:1 in P. dikowi, 2:1 in P. hennigi and 1:1 in P. orientalis.



Figures 1–9. *Perochaeta cuirassa* and *Perochaeta lobo S*. *Perochaeta cuirassa*: **1** 4th sternite, ventral **2** hypopygium, dorsal **3** hypopygium, lateral **4** surstylus, lateral (inward facing) **5** pleural pruinosity pattern, lateral. *Perochaeta lobo*: **6** 4th sternite, ventral **7** hypopygium, dorsal **8** hypopygium, ventral **9** hypopygium, lateral. Scale bars: 0.5mm.

Description (male). Colour. Head capsule mostly black except for thin yellow strip along subgena and parafacial area. Lunule, facial carina and antennae light brown; antennal groove dark brown. Proboscis brown. Thorax, scutellum and abdomen wholly black. Legs largely yellow except for the following: basal regions of fore coxa brown, mid and rear femora with a dark half-ring subapically (edges of which are diffuse on the apical edge), basal half of mid and rear tibiae dark brown. All tarsi with tarsomeres 3–5 brown; tarsomeres 1–2 yellow with brown region apically. Wing clear except for basicostal cell and basal third of costal cell, which is brown. Veins

dark brown. Calypter creamy, margin and fringe-hairs yellowish. Haltere milky yellow with brown base.

Head. Roundish, facial carina short and shallow, facial area receding. Gena and parafacial region narrow. Ocellar prominence and occipital region lightly microtomentose. Chaetotaxy: 1 *ocellar*, 1 divergent *postocellar*, 1 *outer vertical*; *inner vertical* absent. *Orbital* very reduced to absent. 2 *vibrissae.* 3–4 *postocular*. Lower fascial margin lined with setulae.

Thorax. Scutum, postpronotum, scutellum and subscutellum wholly microtomentose. Mediotergite microtomentose but glossy in the medial region. Scutellum twice wide as long. Pleural pruinosity pattern (Fig. 5): Proepisternum glossy with ventral region microtomentose. Anepisternum largely glossy with anterioventral region densely microtomentose. Katepisternum largely with dense tomentosity except for glossy anterioventral region. Anterior side of anepimeron glossy while posterior side lightly microtomentose and posterioventral region densely microtomentose. Postpronotum, katatergite, meron and metepimeron lightly-dusted. Chaetotaxy: 1 *apical scutellar*, 1 reduced, setulae-like *basal scutellar*, 1 *dorsocentral*, 1 *postalar*, 1 *supraalar*, 2 *notopleural*, 1 *postpronotal*, 1 *anepisternal* and 1 *posterior spiracular*. Postpronotoum, prescutum and anepisternum with few, sporadic setulae.

Legs. Forelegs unmodified in males; all femora and tibiae without posteriodorsal or anteriodorsal setae. Mid tibia with row of short setae on anterior apex. Rear tibia with barely-visible osomoterial patch on medial posteriodorsal side. Rear basitarsus with three ventral dark spines basally.

Wings. Without pterostigma. Veins bare. Covered with microtrichiae except for costal, subcostal, basal-medial and posterior-cubital cells, as well as base of cells r1 and r2+3. Microtrichiae sparse on basal region of basal-radial and discal-medial cells. Radial-medial cross-vein divides discal-medial cell by ratio of 3 : 1. Length: 4.6–4.9 mm.

Abdomen. Glossy black; syntergite 1+2 – tergite 5 normal, tergite 6 missing, syntergite 7+8 present and extending ventrad as a narrow sclerite. Spiracles 1–4 on intersegmental membrane, spiracle 5 on ventral margin of tergite 5, spiracle 7 and 8 adjacent on margin of syntergite 7+8. Sternite 1 broadly rectangular while sternite 2 is triangular, tapering posteriorly; sternite 3 is oblong. 4th sternite heavily modified (Fig. 1); greatly expanded, long as is wide, and in the shape of a breastplate. Two stout moveable appendages (= sternite brushes) branch off laterally; each appendage resembles a painter's brush, with large, thick bristles on the outer side and shorter, thinner bristles on the inner side. Posterior edge of 4th sternite invaginated with strong setae lining the outer discal margin as well as submedially. A Y-shaped apodeme extends underneath and anterior to the 4th sternite.

Hypopygium (Figs 2–4). Cercal plate with two very weak lobes; distal margin of each lobe covered with numerous setae. Hypopygium triangular with a two tooth-like projections on the inner side basal to where the surstylus branches off (Fig. 4). Surstylus itself fused to hypopygium, angled dorsally, and branches off subterminally (Fig. 3). Each surstylus has a large hook-like median projection that curves dorsally and decussates. Terminal section of surstylus shaped like a scapula, with cuticular "teeth" and

setulae on distal margin, and a small inward-facing "tooth" on both the ventral and dorsal region subterminally pointing towards the median.

Distribution. Vietnam (Lào Cai).

Perochaeta lobo Ang 2010, sp. n.

urn:lsid:zoobank.org:act:5B7C6CFA-9DCC-4377-9893-CBD789E333E9 Figs 6–9

Material. *Holotype.* ♂ (RMBR), **Vietnam**, Lào Cai Province, Sa Pa Valley. Baited with cow dung at forest edge next to a small cascade alongside highway, ca. 850m along the road westward of the Thác bạc (Silver Waterfall) tourist attraction [22°23'23.90N 103°44'50.32E, elevation 2600m above sea level, ASL]. Collected 16.VII.2010 (Ang Y).

Etymology. The specific epithet is a phonetic translation of Greek " $\lambda \alpha \beta \dot{\alpha}$ ", which refers to the large, distinct lobe found on each lateral half on the posterior margin of the 4th sternite.

Diagnosis. The adult male is very similar to *P. cuirassa* and can only be reliably distinguished based on the 4th sternite and hypopygium. The 4th sternite [cf. *P. lobo* (Fig. 6) and *P. cuirassa* (Fig. 1)] can be distinguished to species by the presence of distinct lobes on the posterior end, the long, thin sternite brush (as opposed to short and squat in *P. cuirassa*), and the sternite itself being narrower (half wide as long). The structure of the hypopygium (Figs 7–9) is also diagnostic given that it is the only *Perochaeta* with a surstylus that has a dorsal flap along its length and a long distal-pointing projection sub-basally. Other diagnostic characters that distinguish *P. lobo* from *P. dikowi*, *P. hennigi* and *P. orientalis* as described in diagnosis for *P. cuirassa*.

Description (male). Colour. As described in *P. cuirassa* except for fore and rear basitarsi, which are brown with yellow base, mid basitarsus yellow with slight brown region apically.

Head. As described in P. cuirassa.

Thorax. As described in P. cuirassa; pleural pruinosity pattern as in Fig. 5.

Legs. Forelegs unmodified; as described in P. cuirassa.

Wings. Coloration, venation and microtrichia distribution as described in *P. cui*rassa. Length: 4.2 mm.

Abdomen. Tergites and sternites 1–3 as described in *P. cuirassa*. Sternite 4 heavily modified (Fig. 6); almost twice long as wide and raised from the abdomen. Posterior edge of 4^{th} sternite deeply invaginated and raised to form two large distinct lobes densely populated with strong bristles, mainly lining the outer discal margin. Two long, thin moveable appendages (= sternite brushes) branch off laterally on the posterior end of the sclerite, each with large, thick bristles on the outer region and some shorter, thinner bristles closer to the inside. A Y-shaped apodeme extends underneath and anterior to the 4^{th} sternite.

Hypopygium (Figs 7–9). Cercal plate with two very weak lobes; distal margin covered with numerous setae. Hypopygium triangular with bicuspid projection before the



Figures 10–14. Various *Perochaeta* 4th sternites and hypopygia. *Perochaeta hennigi* 3° redrawn from Ozerov (1992): **10** 4th sternite, ventral **11** hypopygium (half), dorsal. *Perochaeta dikowi* 3° redrawn from Ang et al. (2008): **12** 4th sternite, ventral **13** hypopygium (half), dorsal. *Perochaeta orientalis* 3° redrawn from Duda (1926): **14** hypopygium, lateral.

base of the surstylus. Surstylus itself fused to hypopygium and branches off terminally, with a dorsal flap along its length and a longish distal-pointing projection sub-basally. Terminal section of surstylus shaped like a scapula, with distal-pointing cuticular "teeth" and setulae on distal margin, and a very small inward-facing "tooth" on both the ventral and dorsal region subterminally pointing towards the median.

Distribution. Vietnam (Lào Cai).

Sepsis monostigma Thompson, 1869

Figs 15-17

Sepsis monostigma Thompson, 1869. Kongliga svenska fregatten Eugenies resa omkring Jorden, 2(1): 443.

Material. $\partial \partial Q Q$ (RMBR), **Vietnam**, Lào Cai Province, Sa Pa Valley. From ex culture established from Q collected from dung on cow farm, 4km NE of Sa Pa town [22°21'28.19"N 103°51'53.35"E, elevation 1250m ASL]. Collected 15.VII.2010 (Ang Y).

Taxonomic remarks. Sepsis monostigma is an Oriental species that resembles Sepsis pseudomonostigma Ursu, 1969 but is geographically exclusive from S. pseudomonostigma (which has only been recorded in South and South-east Europe and Central Asia). Sepsis monostigma can be differentiated by the two long medioventral spines (one short spine in S. pseudomonostigma) on the fore femur (Fig. 15), lack of ventromedial spinules on the fore tibia (Fig. 16) and surstylus (Fig. 17) being much thinner than that in S. pseudomonostigma.

Distribution. China (Guandong), Taiwan, India (Jammu and Kashmir, Uttar Pradesh, West Bengal), Japan (Hokkaido Is., Honshu Is., Kyushu Is.), Korea, Philippines (Luzon Is.), Sri Lanka, South Russia (Amurskaya Oblast', Irkutskaya Oblast', Khabarovskiy Kray, Krasnoyarskiy Kray, Novosibirskaya Oblast', Primorskiy Kray, Sakhalinskaya Oblast'), Vietnam (Lào Cai).



Figures 15–23. Various *Sepsis* forelegs and hypopygia. *Sepsis monostigma*: 15 fore femur, posterior 16 fore tibia, posterior 17 hypopygium, dorsal. *Sepsis sepsi*: 18 fore femur, posterior 19 fore tibia, posterior 20 hypopygium, dorsal. *Sepsis nitens*: 21 fore femur, posterior 22 fore tibia, posterior 23 hypopygium, dorsal. Scale bars: 0.5mm.

Sepsis sepsi Ozerov, 2003

Figs 18-20

Sepsis sepsi Ozerov 2003. Zoologicheskiy zhurnal, 82, 10: 1276.

Material. $\Im \Im \Im \Im$ (RMBR), **Vietnam**, Ha Tay Province, Ba Vi National Park. From ex culture established from \Im collected from rubbish dump near temple at summit of mountain [21°3'45.84"N 105°21'57.63"E, elevation 800m ASL]. Collected 11.VII.2010 (Ang Y). 2 \Im (RMBR), **Indonesia**, West Sumatra, Bukit Tingei Regency, Tanjung Mutiara Dist., Bantar Gadang Beach, [0°24.792"S 99°56.307"E 0m ASL]. Collected 6.VII.2007 (Lohman D).

Taxonomic remarks. Sepsis sepsi bears some resemblance to *S. nitens* and was initially thought to be such by Hennig but later identified it as a 'Sepsis n. sp.' (1941), and was formally described by Ozerov (2003). Sepsis sepsi can be distinguished from *S. nitens* based on the row of four large spines on a large rounded ventromedial bump of the fore femur (Fig. 18) as opposed to three spines arranged triangularly on a slightly proclinate bump in *S. nitens* (Fig. 21). The fore tibia (Fig. 19) lacks a rounded lobe present in *S. nitens* (Fig. 22), and the surstylus differs in structure [cf. *S. sepsi* (Fig. 20) and *S. nitens* (Fig. 23)].

Distribution. Indonesia (Sumatra, Sumbawa), Vietnam (Ha Tay).

Sepsis spura Ang 2010, sp. n.

urn:lsid:zoobank.org:act:581DEFE8-8274-4365-BCDF-7F34BB59A258 Figs 24–31

Material. *Holotype*. ♂ (RMBR), Vietnam, Lào Cai Province, Sa Pa Valley. Collected from dung on cow farm, 4km NE of Sa Pa town [22°21'28.19"N 103°51'53.35"E, elevation 1250m ASL]. Collected 15.VII.2010 (Ang Y). *Paratypes.* 2 ♂ (RMBR), Indonesia, West Sumatra, Bukit Tingei Regency, Tanjung Mutiara Dist., Bantar Gadang Beach, [0°24.792"S 99°56.307"E 0m ASL]. Collected 6.VII.2007 (Lohman D). ♂ (RMBR), Indonesia, N. Sulawesi, Tondano Province, Kampung Jawa [1°17'18.11"N, 124°52'30.05"E, elevation 650m ASL]. Collected 12.V.2009 (Ang Y).

Etymology. The specific epithet old English for "spur", and refers to the distinct spur-like medioventral tubercle found on the male fore femur.

Diagnosis. Adult males of Sepsis spura closely resemble Sepsis nitens but can be distinguished by the following characters: (1) Medioventral tubercle on male fore femur of S. spura is spur-like and bent at a forward angle with two smaller adjacent spines dorsally positioned at the end of the tubercle and one larger spine at the ventral end (Figs 24, 26), while the tubercle in S. nitens is thicker on the base and has its three spines positioned more in a anterio-posterior fashion (Fig. 21). (2) The basal laminalike projection on the fore tibia of S. spura (Figs 25, 27) merges back with the tibia gently, but ends off with a distinct lobe in S. nitens (Fig. 22). The short spines found posteriorly on the projection are also much weaker than those found in S. nitens. (3) Sepsis spura (Figs 25, 27) has only one anterior lamina-like protrusion on the distal portion of the fore tibia, while S. nitens (Fig. 22) has such protrusions on both anterior and posterior sides. (4) The surstylus of S. nitens (Fig. 23) has a rather angular basal swelling and is relatively straight, curved only at the terminus, while the surstyli of S. spura (Figs 30, 31) has a rounded basal swelling and is medially curved for the entirety of the surstylus. Sepsis spura can be distinguished from other Sepsis, also based on the specific structure of the male fore leg ornamentation and the shape of the surstylus.

Description (male). *Colour.* Head capsule mostly brown with a thin light brown strip on gena; fascial margin black. Vertex dark brown. Facial carina and lunule light grey-brown. Pedicel dark brown, 1st flagellomere yellowish, arista brown. Proboscis whit-



Figures 24–31. *Sepsis spura.* **24** fore femur, posterior **25** fore tibia, posterior **26** fore femur, anterior **27** fore tibia, anterior **28** pleural pruinosity pattern, lateral **29** 4th and 5th sternites, ventral **30** hypopygium, dorsal **31** hypopygium, lateral. Scale bars: 0.5mm.

ish yellow. Forelegs wholly yellow. Mid and rear coxa yellow with brown base. Mid femur brown but yellow on basal and distal tips; mid tibia brown on basal half and diffuses to yellow on apical half. Rear femur yellow but brown on dorsal region, while rear tibia wholly brown. Fore tarsus with tarsomeres 3–5 brown, mid tarsus with tarsomeres 3–5 very lightly brown, rear tarsus with tarsomeres 4 and 5 brown. Wing clear except for basicostal cell and basal region of costal cell, which is light brown. Veins dark brown. Calypter clear, margin and fringe-hairs yellowish. Haltere white. Thorax mostly dark brown, but pronotopleuron is yellow. Abdominal tergites and sternites glossy dark brown.

Head. Roundish, facial carina short and shallow, facial area receding. Gena and parafacial region narrow. Largely glossy except for lightly microtomentose occipital region. Chaetotaxy: 1 *ocellar*, 1 divergent *postocellar* (*ocellar* longer than *postocellar*), 1 *inner vertical*, 1 *outer vertical* (*outer* subequal to *inner*). *Orbital* very reduced to absent. 3–4 vibrissae. 3–4 postocular. Lower fascial margin lined with setulae.

Thorax. Scutum, postpronotum and scutellum wholly microtomentose. Subscutellum microtomentose with a small glossy spot ventromedially. Mediotergite microtomentose on margins and glossy in the medial region. Scutellum twice wide as long. Pleural pruinosity pattern (Fig. 28): Proepisternum lightly microtomentose. Anepisternum largely glossy with a small strip on the anterioventral and posteriodorsal margins very lightly dusted. Balsare glossy. Katepisternum densely microtomentose.

Anepimeron glossy. Katatergite, meron and metepimeron microtomentose. Chaetotaxy: 1 *apical scutellar*, 1 reduced *basal scutellar*, 1 row *dorsocentral* with posterior-most two setae as bristles, 1 row *acrostichial* and 1 *postalar*, 1 *supraalar*, 1 *notopleural*, 1 *postpronotal*, 1 *anepisternal* and 2 *posterior spiracular*.

Legs. Forelegs modified; fore femur (Figs 24, 26) with large submedian ventral spine and robust, forward curving spur-like protrusion at the median. This protrusion terminates with three short stout spines, with two adjacently positioned dorsally and one positioned ventrally. Fore tibia (Figs 25, 27) with a submedial and medial cuticular laminar extension; submedial extension with row of short spines. Additional chaeto-taxy: Fore tibia with apical *anteriodorsal*. Mid femur with 1 *anterior*. Mid tibia with 2 *posterior* on median and subapex, 2 *dorsal* on basal 2/3 and apex, 1 *anterior* on apex, 1 *anterioventral* on basal 2/3 and 1 *ventral* on apex. Hind femur with 1 *anteriodorsal* at median and subapically. I *anterior* at median and 2 *anterioventral* medially and subapically. Rear basitarsus with 2 ventral dark spines basally.

Wings. Without pterostigma. Veins bare. Covered with microtrichiae except for basal half of basal-medial cell. Anterior region of r2+3 cell with sparse microtrichiae. Radial-medial cross-vein divides discal-medial cell by ratio of slightly less than 2 : 1. Length: 2.9–3.5mm.

Abdomen. Tergites glossy black, syntergite 1+2 – tergite 5 normal, tergite 6 missing, syntergite 7+8 present and extending ventrad as a narrow sclerite. Spiracles 1–3 on intersegmental membrane close to tergite, spiracles 4 and 5 within respective tergites near margin. Spiracles 7 and 8 adjacent on margin of syntergite 7+8. Sternite 1 a broad rectangle with posterior invaginations while sternite 2 is triangular, tapering posteriorly; sternite 3 is oblong. Sternite 4 is V-shaped with setae and one stout discal bristle on each lateral margin; sternite 5 reduced to a thin lateral crescent (Fig. 29).

Hypopygium. Cercal plate with two very weak lobes, each with single distal setae. Hypopygium and surstylus relatively setaeless. Surstylus fused to hypopygium; bulging medially at base but thin and curved medially, slightly dentate terminally (Figs 30, 31).

Distribution. Indonesia (Sulawesi, Sumatra), Vietnam (Lào Cai).

Key to species of the genus *Perochaeta* Duda, 1926 (males)

1	Ratio of radial-medial cross-vein dividing discal-medial cell is 1:12
_	1:2 to 1:3 3
2	Surstylus without large medial protrusions; with small dentate terminus
_	Sternite brush embedded in membrane
3	Sternite brush as a free sclerotized lobe
_	Sternite brush with few bristles (ca. 5 short and 5 long bristles); surstylus with
	large, squat triangular protrusion on inward median
	Perochaeta hennigi Ozerov, 1992

4	Surstylus without any large medial protrusions
_	Surstylus with large medial protrusions7
5	Sternite brush with few bristles (ca. 5 short bristles and 5 long bristles); sursty-
	lus without large median protrusionsPerochaeta dikowi Ang et al., 2008
_	Medial protrusion emerges dorsally on surstylus; posterior margin of 4 th ster-
	nite V-shaped with many bristles Perochaeta cuirassa Ang, 2010
6	Medial protrusion emerges ventrally on surstylus; posterior margin of 4th ster-
	nite as two distinct lobes with many bristles Perochaeta lobo Ang, 2010

Discussion

Notes on Perochaeta

Perochaeta cuirassa and P. lobo are the first Perochaeta species from Vietnam. That they are found in the same locality is surprising, given that Perochaeta is a small Oriental genus with only three described species. As discussed in Ang et al. (2008), the species in this genus appear to be only found in mid- and high-elevation localities in Asia above 650m ASL: Perochaeta dikowi was found on Fraser's Hill, Malaysia (1300m ASL; Ang et al. 2008), P. hennigi is only known from Thawalamtenne, Sri Lanka (670m ASL; Ozerov 1992) and Tamil Nadu, India (1200m and 1400m ASL; Iwasa and Tewari 1994). The type locality of *P. orientalis* is the Jiji Township in Taiwan ("Chip-chip" as designated by de Meijere; Duda 1926) which is within the Chung Yang mountain range that has an average elevation >1000m ASL, and can also found in regions such as Indonesia (Seram Is., 750m; Iwasa 2001). The new species P. cuirassa and P. lobo were found at 2600m ASL. A male Perochaeta specimen from Flores Island, Indonesia, examined by Hennig (1941) was also collected from a mid elevation site (1200m ASL). Unfortunately, the latter was poorly preserved and could not be described, but based on Hennig's illustration, it likely constitutes a new species. Given that Perochaeta species are restricted to elevated sites, we predict that the number of species will rapidly grow as more mid and high elevation localities are sampled.

Adding two new species to *Perochaeta* is also of interest, because this (small) genus is very atypical for sepsids. Males of most sepsids have modified forelegs (e.g., cuticular tubercles and stout, enlarged spines; see Figs 15–16, 18–19, 21–22, 24–27) which are used to grab the base of female wings during mating (Pont and Meier 2002, Ingram et al. 2008, Puniamoorthy et al. 2008, 2009). However, *Perochaeta* has secondarily reduced foreleg armature, and at least *P. dikowi* has evolved a novel mounting behavior that does not involve the foreleg grasp (Ang et al. 2008). This has made *Perochaeta* a model for testing the correlation between the evolution of behavior and morphology (see Puniamoorthy et al. 2008, 2009).

Notes on Sepsis

Sepsis spura appears to be a relatively widespread species ranging between Indonesia and Vietnam, and it can be found across all elevations, ranging from highlands (Sa Pa Valley, Vietnam; 1250m ASL) through mid elevation areas (Kampung Jawa, Indonesia; 650m ASL) to sea level (Bandar Gadang Beach, Indonesia). Such widespread species of insects are relatively common in Southeast Asia and are increasingly attracting attention because they can give rise to new species (see Balke et al. 2009). At all localities the *S. spura* is found in low numbers on bovine dung while other *Sepsis* species can be very abundant. This, along with its morphological resemblance to *S. nitens*, may explain why *S. spura* is only described now. It is likely to belong to a clade of *Sepsis* species without wingspots (see Su et al. 2008).

Sepsis sepsi was first described from Sumbawa Is., Indonesia at 450m ASL (Ozerov 2003), but has subsequently been collected at low elevation sites in Sumatra as well as now at mid-elevation sites (800m ASL) in Ba Vi, Vietnam. This suggests that *Sepsis sepsi* is a relatively widespread species able to live in low to mid elevation habitats. We predict that it is also likely to be found in other areas between Indonesia and Vietnam. The record of the widely distributed *Sepsis monostigma* in Vietnam is not unexpected given that it falls within the recorded range from India to the far east of Russia.

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RESEARCH ARTICLE



Revision of Nearctic species of Esagonatopus, with description of a new species from Florida (Hymenoptera, Dryinidae)

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Abstract

Esagonatopus floridensis **sp. n.** is described from Florida, Oklaloosa County (USA). A revision of the three Nearctic species of *Esagonatopus* Olmi, 1984 is presented. New data on geographic distribution, morphologic variability and opposite sexes of *E. niger* (Fenton, 1924) and *E. perdebilis* (Perkins, 1907) are given. A key to the Nearctic species of *Esagonatopus* is presented.

Keywords

Taxonomy, Esagonatopus floridensis, USA, key, Dryinidae

Introduction

Dryinidae (Hymenoptera: Chrysidoidea) are parasitoids of Hemiptera Auchenorrhyncha (Guglielmino and Olmi 1997, 2006, 2007). *Esagonatopus* Olmi, 1984 is a genus present in the Nearctic and Neotropical regions and belonging to the subfamily Gonatopodinae. Six species of *Esagonatopus* have been described from the Americas (Olmi 1984, 1986; Virla 1997; Virla and Olmi 2007) and the genus was revised by Olmi (1984) and Virla and Olmi (2007). A key to the four Neotropical species of *Esagonato-pus* was presented by Virla and Olmi (2007).

In 2009 and 2010 we have examined additional specimens of *Esagonatopus* from the United States, Canada and Mexico and have found a new species described herein. This material made it possible to revise the entire group of Nearctic species and provide new data on geographical distribution, morphologic variability and opposite sexes of *E. niger* (Fenton, 1924) and *E. perdebilis* (Perkins, 1907).

Material and methods

The descriptions follow the terminology used by Olmi (1984, 1994, 1999). The measurements reported are relative, except for the total length (head to abdominal tip, without the antennae), which is expressed in millimetres. In the descriptions, POL is the distance between the inner edges of the lateral ocelli; OL is the distance between the inner edges of a lateral ocellus and the median ocellus; OOL is the distance from the outer edge of a lateral ocellus to the compound eye; OPL is the distance from the posterior edge of a lateral ocellus to the occipital carina; TL is the distance from the posterior edge of an eye to the occipital carina.

The treatments of *E. niger* and *E. perdebilis* are updated by adding new localities and morphological variations to the descriptions reported by Olmi (1984).

In the figures of male genitalia the right half was removed.

In the text ! means that the specimen was examined personally by the authors.

The specimens studied in this paper are deposited in the following collections:

AMNH	American Museum of Natural History, New York, U.S.A.
BNC	Benoît Nusillard's collection, Montboucher sur Jabron (France).
BPBM	Bernice P. Bishop Museum, Honolulu, Hawaii, U.S.A.
CDAE	California State Collection of Arthropods, Department of Food and Agri-
	culture, Sacramento, California, U.S.A.
CNC	Canadian National Collection of Insects, Ottawa, Canada.
DEUK	Department of Entomology, College of Agriculture, University of Ken-
	tucky, Lexington, Kentucky, U.S.A.
EMG	Entomology Museum, University of Georgia, Athens, Georgia, U.S.A.
LACM	Natural History Museum of Los Angeles County, Los Angeles, California,
	U.S.A.
MOLC	Massimo Olmi's collection, c/o Department of Plant Protection, University
	of Tuscia, Viterbo, Italy.
MZLU	Zoological Institute, Lund, Sweden.

PMA	Provincial Museum of Alberta, Edmonton, Alberta, Canada.
RDHC	Robert D. Haines' collection, Visalia, California, U.S.A.
SEMC	Natural History Museum, University of Kansas, Lawrence, Kansas, U.S.A.
TAMU	Department of Entomology, Texas A. & M. University, College Station,
	Texas, U.S.A.
UCR	Department of Entomology, University of California, Riverside, California,
	U.S.A.
USNM	National Museum of Natural History, Washington, D.C., U.S.A.

Systematic Accounts

Genus Esagonatopus Olmi, 1984

Esagonatopus Olmi, 1984: 1493. Type species: *Esagonatopus niger* (Fenton, 1924), orig. desig.

Diagnosis. Female: apterous; pronotum crossed by a strong transverse impression; enlarged claw with distal apex pointed, with a small subapical tooth, without lamellae, with bristles or peg-like hairs; antenna without rhinaria (sensu Olmi 1984); palpal formula 6/2; tibial spurs 1/0/1. Male: fully winged; occipital carina absent or incomplete and only present behind and shortly on the sides of posterior ocelli; occiput concave; temples present; palpal formula 6/2; tibial spurs 1/1/2.

Distribution. Nearctic, Neotropical. **Hosts.** Cicadellidae (Guglielmino and Olmi 2006) **Species.** Six.

Key to the Nearctic species of Esagonatopus

Females

1	Meso-metapleural suture distinct and complete	floridensis sp. n.
_	Meso-metapleural suture obsolete	
2	Metanotum with lateral rounded protrusions (Fig. 1)	niger (Fenton)
_	Metanotum laterally not protruding (Fig. 4)pera	lebilis (R. Perkins)

Males (unknown in E. floridensis)

1	Notauli posteriorly separated; dorsal process of paramere	es broader (Fig. 3)
		<i>niger</i> (Fenton)
_	Notauli posteriorly meeting; dorsal process of paramet	res very slender and
	narrow (Fig. 6) <i>per</i>	debilis (R. Perkins)

Esagonatopus niger (Fenton)

Figs 1, 2, 3

Chalcogonatopus nigrus Fenton 1924: 193. Neogonatopus niger (Fenton): Freytag 1980: 145. Esagonatopus niger (Fenton): Olmi 1984: 1495. Esagonatopus perdebilis (Perkins): Malausa et al. 2003: 25. Esagonatopus perdebilis (Perkins): Nusillard et al. 2003: 3. Esagonatopus perdebilis (Perkins): Malausa 2004: 23. Esagonatopus niger (Fenton): Malausa 2004: 23. Esagonatopus niger (Fenton): Guglielmino and Olmi 2006: 53, 54. Esagonatopus niger (Fenton): Moya-Raygoza and Olmi 2010: 99.

Material examined. Type: *Holotype*, female, USA: Iowa, Story Co., Ames, 8.vii.1923, C.J. Drake coll., ex *Scaphoideus* sp. probably *immistus* Say (USNM!). Further specimens examined: CANADA: Ontario: Marmora (CNC!); Near Windsor, Ojibway Park (PMA!); Ottawa (MZLU!); Walpole Island (MZLU!); S Minonico (CNC!); St. Davids (CNC! AMNH!). MEXICO: Mexico (New record): Chapultepec (USNM!). Morelos: Cuernavaca (USNM!). U SA.: Arizona: Johnson Co., McKay Bog, 9 mi. NE Clarksville (CNC!). California (New record): Tulare Co., Ash Mountain, Kaweah Power Station (RDHC!); Contra Costa Co., Moraga (CDAE!); Imperial Co., Niland (USNM!); Riverside Co., Menifee Valley, 33°39'N 117°13'W (UCR!). Florida (New record): Liberty Co., Torreya State Park (CNC!). Georgia (New record): Rabun Co., Clayton (LACM!). Kentucky: Fayette Co., Lexington (DEUK!); Robertson Co. (Freytag, 1977). New York: Schuyler Co., Valois (BNC!); Yates Co., Dresden (BNC!); Ontario Co., Geneva (BNC!). North Dakota: Walsh



Figures 1–3. *Esagonatopus niger.* I Scutum and metathorax + propodeum (in dorsal view) of a female specimen from Mexico, Cuernavaca **2** Chela of holotype **3** Male genitalia of a specimen from New York, Geneva. Scale bar 0.31 mm for 1, 0.12 mm for 2 and 0.06 mm for 3.

Co., Grafton (AMNH!); Ramsey Co. (AMNH!). **Pennsylvania:** Dauphin Co., Harrisburg (USNM!). **Virginia (New record):** Louisa Co., 6.5 Km S of Cuckoo (AMNH!).

Diagnosis. Female with meso-metapleural suture obsolete; metanotum with lateral rounded protrusions (Fig. 1). Male with notauli posteriorly separated; dorsal process of parameres broad (Fig. 3).

Redescription. Female: apterous; length 2.3–3.0 mm. Head black or ferruginous, except anterior region of face, clypeus and mandibles yellow; occasionally head completely yellow or with a black transverse band on vertex. Antenna brown, except segment 1 testaceous, or yellow and segments 8-10 darkened; occasionally antennae totally yellow. Mesosoma usually black, occasionally completely yellow, or ferruginous, with irregular dark spots. Petiole black. Gaster usually brown-ferruginous; occasionally testaceous. Legs completely yellowish red, or with fuscous areas. Occasionally body completely yellow or testaceous, with petiole black. Antenna clavate; antennal segments in following proportions: 8:5:15:9:6:6:5:5:5:7. Head excavated, shiny, smooth, with vertex without sculpture and face and occiput granulated; frontal line complete; occipital carina absent; POL = 1.5; OL = 2; OOL = 7.5. Palpal formula 6/2. Pronotum crossed by a strong transverse impression, shiny, with anterior collar smooth and without sculpture, disc granulated and lateral regions sculptured by longitudinal striae. Scutum shiny, with few longitudinal striae, without lateral pointed apophyses. Scutellum flat, shiny, without sculpture. Meso-metapleural suture obsolete. Metanotum transversely striate, not hollow behind scutellum. Metathorax + propodeum shiny, granulated, with lateral rounded protrusions (Fig. 1). Mesopleura, metapleura and posterior surface of propodeum strongly transversely striate. Fore tarsal segments in following proportions: 13:2:5:16:23. Enlarged claw (Fig. 2) with a small subapical tooth and a row of 7–9 peglike bristles. Segment 5 of fore tarsus (Fig. 2) with two rows of approximately 16-17 lamellae or one row of 13-15 lamellae (with proximal lamellae longer than medial and distal lamellae); distal apex with a group of 7-18 lamellae. Tibial spurs 1/0/1.

Male: fully winged; length 2.0 mm. Head black, except mandibles testaceous. Antenna brown. Mesosoma and petiole black. Gaster and legs brown. Antenna hairy, filiform; antennal segments in following proportions: 4:5:7:6:6:6:6:5.5:5.5:10. Antennal segment 3 less than three times as long as broad (2.8). Head shiny, granulated; occiput excavated; temples distinct; frontal line absent; occipital carina absent; POL = 6; OL = 2; OOL = 4. Palpal formula 6/2. Scutum shiny, granulated. Notauli complete, posteriorly separated; minimum distance between notauli shorter than greatest breadth of posterior ocelli (2:3). Scutellum and metanotum shiny, smooth, without sculpture. Propodeum completely reticulate rugose. Forewing hyaline, without dark transverse bands; stigmal vein with distal part longer than proximal part (17:8). Dorsal process of the parameres (Fig. 3) broadened. Tibial spurs 1/1/2.

Hosts: Cicadellidae (Guglielmino and Olmi 2006): in USA, California: *Lycioides amoenus* (Van Duzee)(Guglielmino and Olmi 1997); in USA, Iowa: *Scaphoideus* sp. probably *immistus* Say (Fenton 1924); in USA, Kentucky: *Scaphoideus paludosus* Ball (Freytag 1977, 1980, 1985); in USA, New York: *Scaphoideus titanus* Ball (Malausa et al. 2003; Malausa 2004; Guglielmino and Olmi 2006).

Esagonatopus perdebilis (R. Perkins)

Figs 4, 5, 6

Chalcogonatopus perdebilis R. Perkins 1907: 27. Chalcogonatopus perdebilis R. Perkins: Olmi 1982: 313. Esagonatopus perdebilis (R. Perkins): Olmi 1984: 1497. Nec Esagonatopus perdebilis (R. Perkins): Malausa et al. 2003: 25. Nec Esagonatopus perdebilis (R. Perkins): Nusillard et al. 2003: 3. Nec Esagonatopus perdebilis (R. Perkins): Malausa 2004: 23. Esagonatopus perdebilis (R. Perkins): Guglielmino and Olmi 2006: 53. Esagonatopus perdebilis (R. Perkins): Moya-Raygoza and Olmi 2010: 81, 99.

Material examined. Types: *Lectotype* (designated by Olmi 1982), female, USA: Arizona, Santa Cruz Co., Nogales, 22.ix.1906, A. Koebele coll. (BPBM!); same locality label, female paralectotype (BPBM!). Further specimens examined: MEXICO: Michoacan: 83th Km of road 200 from Lazaro Cardenas to Tecoman, near Huahua (MOLC!). Nayarit: about 10 Km S of San Blas, Matachén, Crocodilario (MOLC!). Nuevo Leon: San Juan, Río San Juan (UCR!). USA.: Georgia (New record): Pike Co.



Figure 4. *Esagonatopus perdebilis*. Female specimen from Arizona, Nogales (from Olmi 1984). Length 3.0 mm.



Figures 5, 6. *Esagonatopus perdebilis.* **5** Chela of a female specimen from Arizona, Nogales **6** Male genitalia of a specimen from Mexico, Michoacan, near Huahua. Scale bar 0.12 mm for 5 and 0.08 mm for 6.

(EMG!). **Kansas (New record):** Douglas Co., University of Kansas Natural History Reserve (SEMC!). **Texas:** Brewster Co., Big Bend Nat. Park, Window Trail (CNC!); Presidio Co., Big Bend Ranch SNA, 2.5 mi. SE La Sauceda (TAMU!); Val Verde Co., Seminole Canyon State Park, Rio Grande Trail (TAMU!); Brazos Co., College Station (TAMU!).

Diagnosis. Female with meso-metapleural suture obsolete; metanotum laterally not protruding (Fig. 4). Male with notauli posteriorly meeting; dorsal process of parameres very slender and narrow (Fig. 6).

Redescription. *Female*: apterous; length 2.9–3.1 mm. Completely yellow-testaceous, except petiole black; antenna yellow, except segments 4–10 or 8–10 darkened. Antenna clavate; antennal segments in following proportions: 7:4.5:11:6:5:5:5:4.5:4:6. Head excavated, shiny, smooth, with vertex without sculpture and anterior region of face and occiput granulated. Palpal formula 6/2. Pronotum crossed by a strong transverse impression, shiny, without sculpture. Scutum shiny, without sculpture, without lateral pointed apophyses. Scutellum flat, without sculpture. Meso-metapleural suture obsolete. Metanotum transversely striate, not hollow behind scutellum. Metathorax + propodeum shiny, without sculpture, without lateral rounded protrusions (Fig. 4). Posterior surface of propodeum strongly transversely striate. Fore tarsal segments in following proportions: 10:2:3:13:19; Enlarged claw (Fig. 5) with a small subapical tooth and a row of 7–9 peg-like bristles. Segment 5 of fore tarsus (Fig. 5) with 1–2 rows of approximately 13–15 lamellae; distal apex with a group of 9–11 lamellae. Tibial spurs 1/0/1.

Male: fully winged; length 1.6 mm. Head black, except mandibles testaceous; antenna brown; mesosoma black; gaster brown-black; legs brown, except articulations, fore tibiae and fore tarsi testaceous. Antenna hairy, filiform; antennal segments in following proportions: 4:4.5:6:5:5:4:4:5:4:7; antennal segment 3 about three times as

long as broad (6:2). Head dull, granulated, laterally with two shiny and smooth areas situated between posterior ocelli and eyes and surrounded by very low keels; frontal line absent; occipital carina absent; occiput concave; temples distinct; POL = 4.5; OL = 2; OOL = 3. Palpal formula 6/2. Scutum dull, granulated. Notauli complete, posteriorly meeting. Scutellum and metanotum shiny, very finely punctate, without sculpture among punctures. Propodeum dull, reticulate rugose, without keels. Forewing hyaline, without dark transverse bands; marginal cell open; distal part of stigmal vein longer than proximal part (10:6). Dorsal process of the parameres (Fig. 6) slender, much shorter than parameres, with distal apex broadened. Tibial spurs 1/1/2.

Hosts: Cicadellidae (Guglielmino and Olmi 2006): in Mexico, Michoacan: *Xyphon sagittifera* (Uhler) (Guglielmino and Olmi 2006; Moya-Raygoza and Olmi 2010). Quotation by Nusillard et al. (2003) and Malausa et al. (2003) in USA, New York, from *Scaphoideus titanus* Ball wrong because related to testaceous females of *Esagonatopus niger* (Fenton).

Esagonatopus floridensis Olmi & Guglielmino, sp. n.

urn:lsid:zoobank.org:act:926B0805-93C4-4D90-B8C7-F08B8C938B26 Figs 7, 8, 9

Etymology. Floridensis from Florida, where this species was collected.

Types. *Holotype*, female, USA: Florida, Oklaloosa Co., 2 mi N Holt, 31.v.1991, J.B. Woolley coll. (TAMU (it will be transferred to USNM)!).

Diagnosis. Female with meso-metapleural suture distinct and complete; metanotum with lateral pointed protrusions. Male unknown.

Description. Female: apterous; length 2.7 mm. Head brown, except anterior region of face, clypeus and mandibles testaceous; antenna brown-testaceous; mesosoma brown, except scutum testaceous-yellow; petiole and gaster black; legs testaceous, except part of coxae and clubs of femora brown. Antenna clavate; antennal segments in following proportions: 9:4:11:6:5:4.5:4:4:5. Head excavated, shiny, smooth, without sculpture, except occiput granulated; frontal line complete; occipital carina incomplete, shortly present behind and on the sides of posterior ocelli; POL = 1; OL =2; OOL = 7; greatest breadth of posterior ocelli about as long as POL. Palpal formula 6/2. Pronotum crossed by a strong transverse impression, shiny, with anterior collar smooth and without sculpture, disc sculptured by weak longitudinal striae. Scutum shiny, with few longitudinal striae, without lateral pointed apophyses. Scutellum flat, shiny, without sculpture. Meso-metapleural suture distinct and complete. Metanotum transversely striate, not hollow behind scutellum, with sides protruding; lateral protrusions pointed (Fig. 7). Metathorax + propodeum (Fig. 8) dull, with anterior surface weakly sculptured by many longitudinal striae; disc with a track of a median longitudinal furrow. Mesopleura, metapleura and posterior surface of propodeum strongly transversely striate. Fore tarsal segments in following proportions: 11:2.5:4:14:22. Enlarged claw (Fig. 9) with a small subapical tooth and a row of 7 peg-like hairs + 1 hair.



Figures 7–9. *Esagonatopus floridensis* sp. n. 7 Mesosoma in dorsal view 8 Mesosoma in lateral view 9 Chela. Scale bar 0.36 mm for 7, 0.38 mm for 8 and 0.14 mm for 9.

Segment 5 of fore tarsus (Fig. 9) with 2 rows of 1 + 13 lamellae (with proximal lamellae longer than medial and distal lamellae); distal apex with a group of about 6 lamellae. Tibial spurs 1/0/1.

Male: unknown. **Hosts:** unknown.

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RESEARCH ARTICLE



Taxonomic revision and phylogenetic position of Osteocephalus festae (Anura, Hylidae) with description of its larva

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Abstract

Osteocephalus festae is an Amazonian species recently resurrected from a synonymy with O. buckleyi. Because few specimens are known, its morphological variation, diagnostic characters, and distribution are poorly understood. Herein we determine its phylogenetic relationships and provide a complete taxonomic account based on recently collected specimens (adults and larvae) from nine localities in Ecuador and Peru. Osteocephalus festae is most similar to O. verruciger from which it differs in having less tuberculate dorsal skin on males, smaller tympanum, and more tooth rows in the oral disk of larvae. A phylogeny based on mitochondrial DNA sequences, genes 12S and ND1, shows that O. festae is closely related to O. buckleyi, O. mutabor and O. verruciger. A clade consisting of O. festae, O. verruciger, and O. buckleyi is characterized by stream dwelling tadpoles. Surprisingly, we found paraphyly among Ecuadorian populations of O. buckleyi and O. verruciger. The causes for paraphyly are unknown but in O. buckleyi may result from the existence of cryptic species.

Keywords

Andes, Amazon, Anura, morphology, Phylogeny, O. buckleyi, O. festae, O. verruciger, tadpole

Introduction

Osteocephalus is a genus of hylinae frogs (tribe Lophiohylini) distributed in the Amazon Basin and the Guiana Shield (Faivovich et al. 2005). There are 24 recognized species of

which half have been resurrected or described since 2000 (Frost 2010). Despite these efforts, taxonomic problems persist, including undescribed species and binomials of unknown validity or poorly understood boundaries. One such case is *O. festae*, a species described by Peracca (1904) on the basis of a single specimen.

The holotype of *O. festae* is an adult female collected at "Valle Santiago", Provincia Morona Santiago, Ecuador. After its description, this binomial was largely ignored until Trueb and Duellman (1971) synonymized it under *O. buckleyi* (Boulenger, 1882) based on comparisons of the holotype of *O. festae* with series of *O. buckleyi* from Guyana, Colombia, Ecuador and Peru. This synonymy was followed by all systematic accounts until Jungfer (2010) correctly resurrected *O. festae* on the basis of the distinctiveness between the holotype of *O. festae* and *O. buckleyi*. Jungfer (2010) also ascribed to *O. festae* five specimens from Napo and Sucumbíos provinces, Ecuador.

Recently collected specimens of *Osteocephalus* from nine populations from southeastern Ecuador and northeastern Peru, one of them at a distance of ~30 km from the type locality (Fig. 1), closely resemble the holotype of *O. festae* and are morphologically and genetically distinctive from other species. They also seem to be distinctive from the specimens ascribed to *O. festae* by Jungfer (2010) which may belong to a different species (see Taxonomic Remarks). Because little is known about *O. festae* beyond the description of its holotype, below we provide an account of its variation, diagnosis, and distribution, as well as a description of its larvae. In addition, we assess its phylogenetic relationships using mitochondrial DNA sequences.

Methods

DNA extraction, amplification, and sequencing

Total DNA was extracted from muscle or liver tissue preserved in 95% ethanol and tissue storage buffer using a guanidine tiocyanate protocol. Polymerase chain reaction (PCR) was used to amplify the mitochondrial genes 12S rRNA and ND1. We amplified one DNA fragment for 12S and one or two overlapping fragments for ND1 using primers listed in Goebel et al. (1999) and Moen and Wiens (2009). PCR amplification was carried out under standard protocols. Amplified products were sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea).

Phylogenetic analyses

A list of the samples included in the phylogenetic analyses is shown in Table 1. For the outgroup, we included sequences of *Osteopilus* and *Hypsiboas* obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank). Outgroup choice was based on



Figure 1. Records of *Osteocephalus festae* (circles), *O. verruciger* (squares), and *O. buckleyi* (triangles). Locality data from Trueb and Duellman (1970) and specimens deposited at the Museo de Zoología of Pontificia Universidad Católica del Ecuador, the Herpetology Collection, Escuela Politécnica Nacional, and CORBIDI (Appendix 1). Numbers correspond to those on Table 1 and Figure 2.

phylogenies showing that *Osteocephalus* is most closely related to *Tepuihyla* and *Osteopilus* (Faivovich et al. 2005 and Wiens et al. 2010). Because missing sequence data can result in misleading estimates of topology and branch lengths in phylogenies

I2S ND1 SBH 266458 Hypsiboas heilprini DQ380357 EU034080 Moen and Wiens 2009; Wiens et al. 2006 USNM 327241 Osteopilus brunneus DQ380382 EU034083 Moen and Wiens 2009; Wiens et al. 2006 SBH 266457 Osteopilus marianae DQ380383 EU034086 Moen and Wiens 2009; Wiens et al. 2006 SBH 191985 Osteopilus marianae DQ380383 EU034087 Moen and Wiens 2009; Wiens et al. 2006 QCAZ 15981 Osteocephalus alboguttatus 1 HQ600629 HQ600596 This study LAC 2216 O. buckleyi 30 DQ380378 EU034082 Moen and Wiens 2009; Wiens et al. 2006 QCAZ 14947 O. buckleyi 17 HQ600628 HQ600595 This study QCAZ 24446 O. buckleyi 13 HQ600633 HQ600600 This study QCAZ 28231 O. buckleyi 15 HQ600634 HQ600606 This study QCAZ 28277 O. buckleyi 15 HQ600639 HQ600606 This study QCAZ 28395 O. buckleyi 16 HQ600661
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OCAZ 27998 O. vasuni 5 HO600637 HO600604 This study

Table 1. Specimens used in the phylogenetic analysis. Numbers correspond to those in the Figures 1 and 2.

(Lemmon et al. 2009), we only included GenBank sequences for which both genes were available. Preliminary sequence alignment was done with CLUSTALW 1.83 (Chenna et al. 2003). The sequence matrix was imported to Mesquite (version 2.72; Maddison and Maddison 2009) and the ambiguously aligned regions were adjusted manually to produce a parsimonious alignment (i.e., informative sites minimized). Phylogenetic trees were obtained using Bayesian inference. The models of character evolution for the Bayesian analyses were chosen using JModelTest version 0.1.1 (Posada 2008) using the Akaike Information Criterion with sample size correction as optimality measure. We applied independent models to each of four partitions: one for 12S and three for each codon position in ND1. Four Markov chains were utilized in each of two Bayesian analyses, the prior for the rate matrix was a uniform dirichlet and all topologies were equally probable a priori. Each analysis ran for $5 \times$ 10⁶ generations. For each analysis, the chain was sampled every 1000 generations. After 5 x 10⁶ generations the average standard deviation of split frequencies was ~ 0.002 indicating that the two analyses have converged into a stationary distribution. The first 50% of sampled trees were discarded as the burn-in and the remaining trees were used for estimating the Bayesian tree, posterior probabilities and other model parameters. Phylogenetic analyses were carried out in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Morphological analyses

For ease of comparison, we generally follow the format of Trueb and Duellman (1971) for diagnosis and description. Morphological terminology and abbreviations follow Lynch and Duellman (1997) for adults and Altig and McDiarmid (1999) for tadpoles. Description of oral disk structure follows Altig and McDiarmid (1999). Notation for hand and foot webbing is based on Myers and Duellman (1982). Sex and reproductive condition was determined by the presence of nuptial pads, vocal sac folds, dorsal skin texture, and/or by gonadal inspection. Tadpoles were staged according to Gosner (1960) and preserved in 10% formalin. Other specimens were fixed in 10% formalin and preserved in 70% ethanol. To identify the tadpoles and juveniles we grew several tadpoles in captivity until they reached the juvenile stage. Juveniles exhibited a color pattern characteristic of *Osteocephalus*. The only other *Osteocephalus* known at the Río Napinaza collection site breeds on ponds (*O. taurinus*) and has a different juvenile morphology (Lima et al. 2006).

Examined specimens (listed in the type-series and Appendix I) are housed at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ), the Herpetology Collection, Escuela Politécnica Nacional (EPN-H), and the collection of the División de Herpetología, Centro de Ornitología y Biodiversidad (CORBIDI).

Principal Components Analysis (PCA) and Discriminant Function Analysis (DFA) were used to assess the degree of morphometric differentiation between adult *O. buckleyi*, *O. festae*, and *O. verruciger* (Werner 1901). Only well preserved specimens

(Simmons 2002) were measured for the following eight morphological variables, following Duellman (1970): (1) Snout-vent length (SVL); (2) head length; (3) head width; (4) tympanum diameter; (5) femur length; (6) tibia length; (7) foot length; and (8) eye diameter. All variables were log-transformed. To remove the effect of covariation with SVL, the PCA and DFA were applied to the residuals from the linear regressions between the seven measured variables and SVL. We applied a multivariate analysis of variance (MANOVA) to test for morphometric differences between sexes. Because we found significant differences in *O. buckleyi*, the PCA and DFA were applied to each sex separately. For the PCA, only components with eigenvalues > 1 were retained. Sample sizes for *O. verruciger* were 23 males, 5 females; *O. festae* 7 males, 18 females; and *O. buckleyi* 25 males, 3 females. Both PCA and DFA were conducted in JMP[®] 8.01 (SAS Institute 2008).

Twelve morphometric variables were measured in tadpoles, following Altig and McDiarmid (1999): (1) total length; (2) body length; (3) body width; (4) body height; (5) tail length; (6) eye diameter; (7) oral disc width; (8) interorbital distance; (9) internarial distance; (10) maximum tail height; (11) tail muscle height; (12) tail muscle width. All measurements (adults and tadpoles) were made using digital calipers (to the nearest 0.01 mm). Larval tooth row formula is abbreviated as LTRF throughout.

Results

Phylogenetic relationships

The models with the best fit and the estimated parameters for each of four partitions for the Bayesian analyses are shown in Table 2. The Bayesian analyses of 1975 characters (1152 bp of ND1, 823 bp of 12S) resulted in a consensus tree showing strong support for an *Osteocephalus* clade (Fig. 2). Within *Osteocephalus* two clades have strong support: (1) *O. buckleyi, O. festae, O. mutabor*, and *O. verruciger*, and (2) *O. alboguttatus, O. taurinus, O. fuscifacies* and *O. planiceps*. A clade excluding *O. mutabor* from (1) is weakly supported (posterior probability = 0.72).

Phylogenetic analysis shows that both *O. buckleyi* and *O. verruciger* are paraphyletic relative to each other. In *O. verruciger*, the population of Pacto Sumaco is more closely related to one of two clades of *O. buckleyi* than to the other populations of *O. verruciger*. Seven populations of *O. buckleyi* are separated in two well-supported clades, one of which is embedded within *O. verruciger*. In contrast, monophyly among populations of *O. mutabor* and *O. festae* is strongly supported. Within *O. festae*, pairwise uncorrected *p*-genetic distances range from 0.001 (Río Napinaza vs. Miasí) to 0.014 (San Francisco vs. Río Lejía). Uncorrected *p*-distances between both clades of *O. buckleyi* range from 0.047 to 0.060. Distances between Pacto Sumaco and the other populations of *O. verruciger* range from 0.015 to 0.018.
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Partition	Best-fit model	AIC score	Ι	G	К	AC	AG	АТ	0G	CT	GT	Α	G	С	Т
12S	GTR + G + I	15762	0.386	0.740	١	0.079	0.281	0.081	0.007	0.522	0.026	0.334	0.183	0.238	0.243
ND1, 1st	HKY + G + I	14527	0.489	1.385	14.22	١	١	١	١	١	١	0.365	0.129	0.240	0.264
position															
ND1, 2nd	GTR + G	6472	ı	0.185	١	0.024	0.589	0.047	0.042	0.274	0.020	0.261	0.096	0.307	0.334
position															
ND1, 3rd	HKY + G + I	3745	0.295	1.617	14.22	ı	ı	ı	ı	١	١	0.397	0.104	0.216	0.280
position															



Figure 2. Bayesian consensus phylogram depicting relationships within *Osteocephalus*. Phylogram derived from analysis of 1975 bp of mtDNA (genes ND1 and 12S). Numbers in parenthesis corresponds to those on Table 1 and Figure 1. Posterior probabilities resulting from Bayesian Markov chain Monte Carlo searches appear above branches. An asterisk represents a value of 1. The outgroup species *Hypsiboas heilprini* is not shown.

Systematic account of Osteocephalus festae

Osteocephalus festae (Peracca, 1904)

- *Hyla festae* Peracca, 1904:39. Holotype: MZUT An208, a female from "Valle Santiago" ("= lower Río Zamora" according to Trueb and Duellman, 1971) Provincia Morona Santiago, Ecuador (Fig. 3G-H).
- Osteocephalus buckleyi (part) Trueb and Duellman, 1971. Synonymy fide Trueb and Duellman, 1971:23.

Diagnosis. Throughout this section, coloration refers to preserved specimens unless otherwise noted. *Osteocephalus festae* is a medium-sized species of *Osteocephalus* having the following combination of characters : (1) size sexually dimorphic; maximum SVL in males 56.1 mm, in females 84.9 mm; (2) skin on dorsum bearing tubercles in males, smooth in females; (3) skin on flanks areolate; (4) hand webbing formula II2½—3¼III3½—2IV; foot webbing formula varying as shown in Table 3 and Fig. 4; (5) dorsum brown, usually with irregular dark marks; (6) venter varying from cream to tan, with ill to well defined brown chocolate blotches; (7) narrow, cream to light brown, labial stripe confluent with similarly colored suborbital mark; (8) flanks cream to light brown with darker reticulations anteriorly and dark blotches posteriorly; (9) dermal roofing bones of the skull weakly exostosed; (10) bones green in life; (11) iris dark brown without reticulations, in life; (12) paired vocal sacs located laterally, behind jaw articulation, (13) in life, juveniles with red iris, and pale elbows, knees, and heels; (14) larvae with LTRF of 4/7 or 5/7.

Osteocephalus festae is most similar to O. verruciger. Both species differ from other Osteocephalus by the combination of a brown iris (in life) and the presence of brown marks in the venter (in life and preservative). Osteocephalus festae differs from O. verruciger in having: (1) less dorsal ornamentation in males (fewer and less developed dorsal tubercles in O. festae), (2) smaller tympanum (1/5 of head length in O. festae vs. 1/4 in O. verruciger), and (3) more tooth rows in larvae (LTRF = 4/7 to 5/7 in O. festae vs. 2/5 in O. verruciger; Fig. 5). Mitochondrial DNA sequences show that O. festae and O. verruciger are not sister species (Fig. 2). Osteocephalus festae differs from O. mutabor Jungfer & Hödl, 2002 in having chocolate blotches in the venter (blotches absent in O. mutabor). It differs from most species of Osteocephalus (except O. verruciger, O. heyeri Lynch, 2002, and O. subtilis Martins & Cardoso, 1987) in having a dark brown iris in life (iris bronze to golden with or without black straight lines or irregular black reticulations in O. alboguttatus (Boulenger, 1882), O. buckleyi, O. cabrerai (Cochran & Goin, 1970), O. deridens Jungfer et al., 2000, O. exophthalmus Smith & Noonan, 2001, O. fuscifacies Jungfer et al., 2000, O. leoniae Jungfer & Lehr, 2001, O. leprieurii (Duméril and Bibron, 1841), O. mutabor, O. oophagus Jungfer & Schiesari, 1995, O. pearsoni (Gaige, 1929), O. phasmatus MacCulloch & Lathrop, 2005, O. planiceps Cope, 1874, O. taurinus Steindachner, 1862, and O. yasuni Ron & Pramuk, 1999). Osteocephalus mimeticus (Melin, 1941) can be discriminated from O. festae in hav-



Figure 3. Dorsolateral and ventral views of *Osteocephalus festae*. **A** QCAZ 41039, adult female, SVL = 79.81 mm **B** Amplectant pair (not collected) from Chonza Alta, Peru **C–D** QCAZ 45674, subadult, SVL = 36.54 mm **E–F** QCAZ 38081, juvenile, SVL = 13.37 mm **G–H** MZUT An208 (holotype), adult female, (SVL = 78.00 mm; Peracca 1904). Holotype photographs by Franco Andreone and photographs of the amplectant pair by PJV. See Appendix I for locality data.

	Hand	Foot
QCAZ 39805 (female)	I basal II2 ¹ /2-3 ¹ /4III3 ¹ /2-2 ⁺ IV	$\mathbf{I}1^{-}-1^{-}\mathbf{II}1^{-}-1^{-}\mathbf{III}1^{-}-1^{-}\mathbf{IV}1^{-}-1^{-}\mathbf{V}$
QCAZ 39809 (female)	I basal II2 ¹ /2—3 ¹ /4III3 ¹ /2—2 ⁺ IV	$I_1 - 1 - I_1 I_1 - 2 - III_1 - 2 - 2 / 2 IV_2 / 2 - 1 - V$
QCAZ 39799 (male)	I basal II2½—3¼III3½—2⁺IV	$I_1 - 1II_1 - 1$ $III_1 - 2\frac{1}{2}IV_2\frac{1}{2} - 1$ V
QCAZ 39802 (female)	I basal II2 ¹ /2—3 ¹ /4III3 ¹ /2—2 ⁺ IV	$I1^{+}-2^{-}II1^{+}-2III1-2IV2-1^{-}V$
QCAZ 26304 (female)	I basal II2½—3¼III3½—2 ⁻ IV	$\mathbf{I}1^{-}-1^{-}\mathbf{II}1^{-}-1^{-}\mathbf{III}1^{-}-1^{-}\mathbf{IV}1^{-}-1^{-}\mathbf{V}$
QCAZ 26488 (male)	I basal II2½—3¼III3½—2 ⁻ IV	$\mathbf{I}1^{-}-1^{-}\mathbf{II}1^{-}-1^{-}\mathbf{III}1^{-}-1^{-}\mathbf{IV}1^{-}-1^{-}\mathbf{V}$

Table 3. Variation in webbing in hand and feet of representative adults of *Osteocephalus festae*. Webbing formula notations follow Savage and Heyer (1967) with modifications by Myers and Duellman (1982).



Figure 4. Ventral views of the right hand and foot of *Osteocephalus festae*. Adult female from Río Napinaza, Ecuador, SVL = 84.93 mm, QCAZ 39811. Hand and foot are shown at the same scale.

ing a black iris with golden marks. Osteocephalus festae differs from O. subtilis and O. heyeri in size (maximum male SVL 38.8 mm in O. subtilis, 36.1 mm in O. heyeri vs. 56.1 mm in O. festae). The presence of areolate skin in the flanks, specially anteriorly, distinguish O. festae from O. leprieurii, O. mutabor, O. pearsoni, O. planiceps, and O. yasuni (smooth to granular skin on flanks; Trueb & Duellman, 1971). Osteocephalus taurinus has weakly areolate skin restricted to the axillary region. Osteocephalus festae further differs from O. buckleyi and O. cabrerai in lacking prominent tarsal tubercles (Jungfer 2010).

Holotype. The holotype is an adult female with SVL = 78 mm (Fig. 3G, H; Peracca, 1904). The descriptions provided by Peracca (1904) and Jungfer (2010) are adequate.

Variation. Variation in dorsal and ventral coloration of preserved specimens is shown in Figures 6 and 7. Dorsal coloration consists of a light brown to dark brown background with irregular marks. There is sexual dimorphism in dorsal tuberculation: in females the dorsum is smooth while in males it varies between having scant



Figure 5. Tadpoles of *Osteocephalus festae* and *O. verruciger*. A–D, G–J are in preservative; E–F in life. A–D: *O. festae*, stage 39, QCAZ 30511; E–F: *O. festae*, stage 33, QCAZ 38074; G–J: *O. verruciger*, stage 36, QCAZ 36751. A, G: dorsal view; B, H: lateral view; C, I: ventral view; D, J: oral apparatus. Photos in life by SRR.

and ill-defined non-keratinized tubercles (most males from Río Napinaza, e.g., QCAZ 26488) to having abundant keratinized tubercles (two males from Chonza Alta, e.g., CORBIDI 758; Fig. 3B).

Ventral surfaces of preserved specimens (Fig. 7) have a cream (QCAZ 39364) to tan (QCAZ 39806) background with darker brown marks that are more distinct and abundant in females (e.g., QCAZ 39811) than in males (e.g., QCAZ 39799); a male from Río Lejía (CORBIDI 623) has an immaculate venter. Ventrally, limbs vary from brown to cream; in QCAZ 39809 and 39811 cream dots are present on hindlimbs; scant cream tubercles can be present in the external edge of the forearm (e.g., QCAZ 39804). The vent region is dark brown to brown bordered by a lighter



Figure 6. Adult *Osteocephalus festae* showing variation in dorsal coloration of preserved specimens. Left to right, upper row: QCAZ 39804, 39811, 39810, 39802, 39798 (females); lower row: QCAZ 39799, 26552, 26488, 26561 (males), 39364 (female). Provincia Loja and Morona Santiago, Ecuador. See Appendix I for locality data.



Figure 7. Ventral views of adult *Osteocephalus festae* showing variation in ventral coloration of preserved specimens. Specimen identity and arrangement is the same as in Figure 6.

area (cream to tan). Flanks are areolate in the anterior half and smooth posteriorly. The areolate portion is cream with dark brown reticulation; the posterior half is cream (e.g., QCAZ39810) to light brown (e.g., QCAZ 39806) with dark brown blotches.

Head shape is rounded in dorsal view and rounded (e.g., QCAZ 39803) to bluntly rounded (e.g., QCAZ 39800–01) in lateral view. Lateral head coloration varies between dark brown (QCAZ 11625) to light brown (QCAZ 39810). Except for QCAZ

TD = Tympar	um Diameter; TL	. = Tibia Length; F	:L = Femur Length.	, All measurements	are in mm.	0		
Species	SVL	FOOT	HL	MH	ED	TD	TL	FL
Males	47.47 ± 5.09	19.38 ± 2.26	14.51 ± 0.99	15.77 ± 1.92	5.01 ± 0.83	2.79 ± 0.29	25.90 ± 1.94	22 ± 6.29
(n = 12)	(38.3 - 56.09)	(16.3-22.34)	(13.1 - 16.46)	(11.5-18.73)	(3.5-6.64)	(2.1-3.06)	(21.7)-28.2)	(20.4-26.06)
Females $(n = 27)$	67.91 ± 10.07 (49.51 -84.93)	29.79 ± 5.13 (20.28-39.99)	20.31 ± 3.20 (15-25.26)	22.60 ± 3.62 (16.1–28.75)	6.25 ± 0.82 (4.4-7.62)	3.72 ± 0.70 (2.1-5.16)	39.13 ± 5.54 (29.08-49.16)	35.35 ± 6.09 (24.3-46.81)
Chonza Alta								
Males	43.72 ± 5.42	17.97 ± 1.95	14.07 ± 1.21	13.82 ± 1.72	4.51 ± 0.95	2.55 ± 0.36	24.03 ± 1.98	22.05 ± 1.47
(n = 4)	(38.3 - 50)	(16.3 - 20.6)	(13.1 - 15.7)	(11.5 - 15.4)	(3.5 - 5.65)	(2.1 - 2.9)	(21.75 - 26.4)	(20.4 - 23.7)
Females	68.72 ± 5.92	29.33 ± 3.29	20.62 ± 3.40	22.33 ± 3.35	6.2 ± 0.89	3.26 ± 0.71	39.56 ± 2.71	34.63 ± 5
(n = 8)	(56.3 - 76.4)	(24.2 - 33.8)	(15-25)	(16.1 - 25.6)	(4.4 - 7.1)	(2.1 - 4.6)	(33.6 - 43.1)	(24.3 - 39.1)
Miasí								
Female	79.81	36.65	21.93	26.12	6.98	3.8	45.73	42.19
(n = 1)								
Río Lejia								
Males	48.3	20.7	14.3	16.8	4.3	2.8	26.8	24.6
(n = 1)								
Females	72.6	30	21.1	23.2	6.1	3.7	41.2	30.4
(n = 1)								
Río Napinaza								
Males	50.82 ± 5.04	21.08 ± 1.02	14.62 ± 0.67	17.4 ± 0.89	5.66 ± 0.66	2.96 ± 0.11	27.67 ± 0.56	24.88 ± 0.80
(n = 4)	(44.47 - 56.09)	(19.94 - 22.34)	(13.96–15.56)	(16.82 - 18.73)	(5.19 - 6.64)	(2.84 - 3.06)	(26.95 - 28.2)	(24.24 - 26.06)
Females	66.37 ± 12.06	29.59 ± 6.10	20.04 ± 3.46	22.53 ± 4.06	6.28 ± 0.87	3.96 ± 0.66	38.35 ± 6.77	35.55 ± 6.89
(n = 16)	(49.51–84.93)	(20.28–38.99)	(15.49–25.26)	(16.75–28.75)	(4.87 - 7.62)	(3.08 - 5.16)	(29.08-49.16)	(25.79-46.81)
San Francisco								
Female	69.54	29.48	19.63	21.73	5.72	3.55	39.63	35.90
(n = 1)								
San Carlos								
Males	47.74 ± 3.09	19.31 ± 0.90	15.00 ± 1.31	15.86 ± 1.13	5.04 ± 0.50	2.87 ± 0.23	25.72 ± 0.85	23.23 ± 0.79
(n=3)	(45.65–51.3)	(18.58–20.32)	(13.89–16.46)	(15.03–17.15)	(4.65–5.61)	(2.60 - 3.06)	(24.76–26.35)	(22.7–24.14)

Table 4. Descriptive statistics for morphometric measurements of adult *Osteocephalus festae*. Mean ± SD is given with range below. Bold figures are averages for individuals of all populations. Abbreviations are: **SVL** = Snout-Vent Length; **FOOT** = Foot Length; **HL** = Head Length; **HW** = Head Width; **ED** = Eve Diameter; 39802 and 41039, there is a lighter (brown to cream) subocular mark. A tan (QCAZ 39805) to cream (QCAZ 39364) labial stripe is always present. The tympanic annulus is concealed dorsally and has lighter color than the background. Variation in hand and foot webbing is shown in Table 3. The distal subarticular tubercle on Finger IV is single in all specimens.

Morphometric data pertain to adults and are summarized in Table 4. In the examined series, the largest male has a SVL of 56.09 mm and the largest female 84.94 mm; mean male SVL = 49.47 mm (n = 12; SD = 5.10), mean female SVL = 67.92 mm (n = 27; SD = 10.08). Females are significantly larger than males (t = 5.52, df = 23, P < 0.001). A MANOVA on the residuals of the regressions between SVL and the other measured variables indicates lack of significant differences between sexes in size-free morphometry (F = 1.052, df = 17, P = 0.433).

Color in life. Based on digital photograph of adult female QCAZ 41039 (Fig. 3A): dorsum dark brown with irregular light brown and yellowish green marks; canthal region dark brown with yellowish green subocular mark and labial band; tympanum brown; flanks greenish brown with dark brown reticulation anteriorly and irregular dark brown marks posteriorly; dorsal surfaces of thighs and shanks dark brown with transversal brown bands bordered with light brown; dorsal surfaces of forelimbs dark brown with irregular brown marks; venter light tan with irregular brown marks; bones green; iris dark brown. Female CORBIDI 761 has a predominantly light brown dorsum with irregular brown marks; clear areas on flanks and below the eye and tympanum are light yellow.

There is significant change in coloration between juveniles and adults. The following description is based on a digital photograph of juvenile QCAZ 38081 (Fig. 3E-F). The dorsum beige with black interorbital band and two large medial ovoid black blotches; flanks dark brown; dorsal surfaces of thighs and shanks brown with cream transversal bars; dorsal surfaces of arms cream, dorsal surfaces of forearms brown with cream transversal bars; knees, elbows, and heels cream; anterior half of the venter cream, posterior half light brown; bones green; iris bright red.

Morphometric comparisons. Three components with eigenvalues > 1.0 were extracted from the PCA for males (Table 5). The three components accounted for 76.4%

		PCA Males]	PCA Female	\$
Variable	PC I	PC II	PC III	PC I	PC II	PC III
Foot length	0.523	0.564	0.242	0.459	0.202	- 0.151
Head length	- 0.243	0.647	0.121	- 0.243	0.394	0.537
Head width	0.407	- 0.430	- 0.208	0.196	0.315	- 0.680
Eye diameter	- 0218	0.259	- 0.673	- 0.061	0.648	- 0.125
Tympanum diameter	- 0.224	0.004	0.655	- 0.435	0.451	- 0.016
Tibia length	0.455	0.407	- 0.020	0.519	0.096	0.285
Femur length	0.441	0.398	- 0.005	0.476	0.266	0.356
Eigenvalue	2.570	1.744	1.030	2.722	1.517	1.382

Table 5. Character loadings and eigenvalues for Principal Components (PC) I–III. The analysis was based seven morphometric variables of adult *Osteocephalus*. Bold figures indicate highest loadings.



Figure 8. Principal components from analysis of seven size-corrected morphological variables. See Table 5 for character loadings on each component.

of the total variation. The highest loadings for the PCA for males were foot length and tibia length for PC I, head length for PC II, and eye diameter and tympanum diameter for PC III (Table 5). The morphometric space of *O. festae* overlaps with *O. verruciger* (Fig. 8) but only slightly with *O. buckleyi*. There are significant differences in PC scores between *O. festae* and *O. buckleyi* along PC II and PC III (t = 2.46, df = 9, P = 0.035; t = 6.76, df = 13, P < 0.001, respectively) but not along PC I (t = 1.61, df = 7, P = 0.115). There are significant differences only along PC III between *O. festae* and *O. verruciger* (t = 9.03, df = 9, P < 0.001).

Three components with eigenvalues > 1.0 were extracted from the PCA for females (Table 5). The three components accounted for 80.3% of the total variation. The highest loadings for the PCA for females were tibia length and femur length for PC I, eye diameter and tympanum diameter for PC II, and head width and head length for PC III (Table 5). There is little overlap in the morphometric space of *O. festae* with *O. verruciger* and *O. buckleyi* (Fig. 8). Principal Components scores are significantly different between *O. festae* and *O. verruciger* along PC I and PC III (t = 4.96, df = 21, P < 0.001; t = 4.91, df = 21, P < 0.001, respectively) but not along PC I and PC III (t = 4.96, df = 21, P = 0.403). Similarly, *O. festae* and *O. buckleyi* differed along PC I and PC III (t = 4.96, df = 21, P = 0.403).

In the DFA classification procedure, 24 out of 25 specimens of *O. festae* were classified correctly. The misclassified female (QCAZ 38420) was assigned to *O. verruciger*. The multivariate analyses (PCA and DFA) show morphometric differentiation between *O. festae* and the closely related *O. verruciger* and *O. buckleyi*.

Tadpoles. Letters in parenthesis refer to individual tadpoles on each lot. The following description is based on lot QCAZ 30511 of ten larvae in Stages 25 (A), 26 (B), 31 (C), 32 (D), 33 (E), 34 (F), 35 (G), 39 (H), 40 (I) and 42 (J). Tadpoles were

asurements of tadpoles of Osteocephalus festae (lot QCAZ 30511). Developmental stages, in parentheses, are defined according to Gosner (1960). Ab-	e: TL = Total Length; BL = Body Length; BW = Body Width; BH = Body Height; TAL = Tail Length; ED = Eye Diameter; ODW = Oral Disc Width;	rbital Distance (measured between center pupils); IND = Internarial Distance (measured between centers of narinal openings); MTH = Maximum Tail
ble 6. Measurements of tadpo	iations are: TL = Total Lengt) = Interorbital Distance (me

))								
					Sta	ge				
Variable	A (25)	B (26)	C (31)	D (32)	E (33)	F (34)	G (35)	H (39)	I (40)	J (42)
TL	30.05	29.49	37.22	35.57	34.75	34.68	39.81	40.39	40.63	40.94
BL	9.51	9.19	11.06	10.07	10.82	11.04	11.44	11.13	11.81	11.16
BW	5.74	5.68	7.25	7.05	7.23	7.10	8.11	7.50	8.46	7.12
BH	4.83	4.98	5.95	5.46	5.87	5.88	6.10	6.32	6.27	4.89
TAL	20.30	20.04	26.59	24.56	24.36	23.76	27.90	28.99	28.69	29.90
ED	1.02	1.30	1.25	1.28	1.47	1.10	1.50	1.41	1.70	1.47
ODW	3.50	3.35	3.90	4.12	3.80	4.43	4.13	4.00	3.94	3.46
IOD	4.27	4.34	5.15	5.40	5.08	5.45	5.04	5.26	5.74	5.02
IND	3.49	3.50	4.01	4.19	3.75	3.94	3.98	4.12	4.01	1.62
MTH	5.30	5.37	5.88	6.10	5.85	6.05	6.70	6.62	6.75	6.06
TMH	2.06	2.00	2.57	2.50	3.26	2.79	3.07	3.07	3.45	2.44
TMW	1.60	1.46	2.70	2.12	2.30	2.50	2.97	2.62	2.78	2.56

collected at Río Napinaza by E. E. Tapia and I. G. Tapia on October 2003. These larvae belong to the exotrophic, benthic guild as defined by McDiarmid and Altig (1999). Morphometric data are provided in Table 6. In dorsal view, a tadpole in Stage 39 (QCAZ 30511H; Fig. 5A) shows elliptical body, widest between eye and spiracle, with rounded snout. Eyes relatively large (body length about 7.89 times larger than eye diameter), directed and positioned dorsolaterally, not visible in ventral view, and separated by a distance 1.27 times the internarial distance. External nares oval, located dorsolaterally, at about one fourth the distance between anterior margin of snout and anterior margin of eye. In profile (Fig. 5B) body depressed (body width/body height = 0.18), flattened ventrally, snout slightly rounded. Oral disc not emarginated. Spiracle sinistral, inner wall free from body, its tip closer to the vent than the eye. Spiracle opening rounded.

Tail musculature robust, decreasing in size towards tip of tail. Dorsal fin not extending onto body, slightly convex and attaining its maximum height at mid length of tail; tail tip rounded; ventral fin convex, beginning at tail-body junction and tapering gradually towards tail tip. Medial vent tube with both walls attached directly to ventral fin, opening directed posteroventrally. Limbs with subarticular patches. Dorsal body, middle body, supraorbital, infraorbital, posterior supraorbital, and posterior infraorbital lateral lines evident. No glands.

Oral disc anteroventral (Fig. 5B; average transverse width 4.35 mm; 58% of body width), not emarginate, LTRF 4/7; papillae distributed around oral disc; tooth rows complete except for medial gap in row A4; A1 = 304, A2 = 322, A3 = 311, A4 = 108 + 112; P1 = 177, P2 = 235, P3 = 234, P4 = 328, P5 = 284, P6 = 330, and P7 = 177.

In preservative, dorsum brown with darker marks between eyes; dark brown dorsolateral stripes extend from mid-body to base of tail; caudal musculature beige with brown spots (Fig 5); skin of flanks, spiracle, vent tube, fins, and around the eyes transparent; belly and fins transparent with white blotches.

Tadpole variation and comparisons with other species. In QCAZ 38074, 26321, 26053, 26498 and 26284 the caudal musculature is cream with brown dots; fins can have dark brown spots without white blotches (e.g., QCAZ 38074, 26321). The LTRF is the same in all stages but in stage 42, rows A2–A4 and P1–P3 have approximately half the number of teeth.

In preservative, ten tadpoles collected in Chonza Alta, Peru, lot CORBIDI-CL-10 in Stages 37 (A-B), 36 (C), 34 (D), 32 (E), 31 (F), 42 (G, H, I) and 44 (J) have dorsum brown with darker marks between the eyes; dark brown dorsolateral stripes from mid-body to base of tail; caudal musculature cream with abundant melanophores; skin of flanks, spiracle, vent tube, and fins transparent; skin around the eyes brown; belly and fins transparent with abundant melanophores. Oral disc with LTRF 5/7; papillae distributed around oral disc; tooth rows complete except for medial gap in row A5.

In life (Fig. 5E-F; QCAZ 38074, stage 33; based on digital photograph), dorsum dark brown with darker marks between the eyes; dark brown dorsolateral lines from mid-body to base of tail; tail musculature light brown with small dark brown melanophores; white dots at tail-body junction; skin is transparent ventrally in anterior half of

body and ventrolaterally in the posterior half; tail musculature light brown with dark brown spots; fins transparent. Iris bronze. Live tadpoles from Chonza Alta (CORBI-DI-CL-10) have dorsum and caudal musculature olive brown; skin transparent ventrally with bright brown flecks, gut visible through the skin; fins translucent brown.

Larvae of *O. verruciger* differ from those of *O. festae* (in parentheses) by being smaller, having uniform dark body (tan to cream), LTRF = 2/5 (4/7 to 5/7); and having a dorsal gap in marginal papillae (marginal papillae complete). For comparison, larvae of *O. verruciger* is shown in Figure 5 G–H. Differences were verified in 30 *O. verruciger* larvae from four localities (lots QCAZ 1579, 10798, 21405 and 36751). Larvae of *O. festae* have the highest number of tooth rows known among *Osteocephalus* (LTRF = 2/3 in *O. oophagus*, 2/6 in *O. buckleyi* and *O. taurinus*; Hero 1990; Jungfer and Schiesari 1995).

Distribution and ecology. Osteocephalus festae has been recorded at nine localities in the Ecuadorian (Loja, Morona Santiago, and Zamora-Chinchipe provinces) and Peruvian Amazon basin (Mariscal Cáceres and Rioja provinces). Localities with known elevation (Río Napinaza, Miasí, San Francisco, Reserva Tapichalaca, Río Lejia, Chonza Alta, Camñopite Bajo, and Naranjillo) range between 1000 and 2200 m of elevation. The elevation at San Francisco (2200 m) is the highest known for Osteocephalus. Maximum airline distance between localities is 440 km. Osteocephalus festae and O. verruciger have similar elevational ranges and seem to replace each other latitudinally in Ecuador (Fig. 1). Records of O. verruciger from Peru (e.g., Trueb and Duellman 1970) are likely misidentified O. mimeticus (Jungfer 2010). Thus, the southernmost confirmed records of O. verruciger are those from Provincia Morona Santiago, Ecuador.

Most of our specimens of *O. festae* are from Río Napinaza, a river surrounded by secondary forest, pastures and agricultural lands. At the collection site, the river has an average width of 2.85 m and an average depth of 23 cm with fast running water and waterfalls that reach 10 m in height (Salazar-Valenzuela 2007). Tadpoles were found in small ponds in the margins of the river. Adults were observed at night next to the river or within the forest on vegetation 40 to 250 cm above the ground.

All the specimens collected in Las Cataratas de Paraiso (Chonza Alta) and Camñopite were found at night on vegetation 50 to 300 cm above the ground, next to fast running streams. Tadpoles (CORBIDI-CL-10) were found in a rocky stream with average width of 4 m and an average depth of 30 to 40 cm with fast running water, close to the base of a waterfall. At both sites the streams are surrounded by secondary forest, pastures and agricultural lands. The specimens from Bajo Naranjillo and Río Lejia were found at night on branches 150 to 200 cm above the ground (in primary forest at Río Lejia and secondary forest surrounded by pastures at Naranjillo).

At Las Cataratas de Paraiso (Chonza Alta), on 1 December 2007, we found twelve males calling from the low vegetation and six amplectant pairs (Fig. 3B). Recently metamorphosed individuals were perching on leaves and rocks at the shore. On 4 November 2008, at the same stream, we only found one adult non-amplectant female, two adult males, several tadpoles, and 12 freshly metamorphosed juveniles on leaves and rocks (e.g., CORBIDI 1962–64). The rainy season in this region generally starts in December but during our surveys heavy rains fell since the first week of November. Three gravid females (CORBIDI 761, SVL 68.6 mm; CORBIDI 762, SVL 69.1 mm; CORBIDI 764, SVL 66.6 mm) contained 1080, 1388 and 1290 eggs respectively. A gravid female CORBIDI 624 (SVL = 71.2 mm) from Río Lejia, collected on 29 January 2008, contained 780 eggs. The color of eggs in preservative is black and brownish-cream.

Vegetation types for Ecuadorian localities (according to the classification of Sierra et al. 1999) are: (1) Evergreen Foothill Forest of the Eastern Slopes of the Southern Andes, characterized by abundant epiphytes, trees reaching 30 m of height with *Po-docarpus* as dominant species (Reserva Tapichalaca), (2) Amazonian Mountain Range Evergreen Foothill Forest, characterized by a mixture of Amazonian and Andean vegetation with a canopy of 30 m (Río Napinaza and Miasí), and (3) Cloud Montane Forest of the Eastern Slopes of the Southern Andes, characterized by trees covered by mosses and abundant epiphytes (San Francisco; Cerón et al. 1999).

Vegetation types of the Peruvian localities (according to Duellman and Pramuk 1999) are Humid Subtropical Forest, characterized by of a variety of moderate to large trees including *Juglans neotropica*, *Cedrela fissipes, Tabebuia*, and genera common in the Amazon lowlands like *Brosimum, Cordia, Inga, Piper*, and *Swietenia*. At some collecting sites, the forest has been cleared for citrus and coffee plantations.

Taxonomic remarks. Jungfer (2010) ascribed two females (EPN 5578, MHNG 2560.60) and three males (EPN 5577, EPN AA-5611, EPN 5607) to *O. festae*. Based on data from Jungfer (2010), both females differ from the holotype and 18 adult females analyzed here (in parentheses) in having a venter uniform tan without marks (brown marks present), webbing almost reaching the ultimate subarticular tubercle in the inner edge of third finger (web reaching half the distance between ultimate and penultimate subarticular tubercles), and larger tympanum size with TD/HL = 0.23–0.25 (TD/HL = 0.14–0.22 among 39 adult males and females). In addition, we did not find the sexual dimorphism in relative tympanum size reported by Jungfer (Student's t = 1.227, df = 23, P = 0.232). Males assigned to *O. festae* also seem to differ from our series in the extent of the axillary membrane (covering half of the upper arm vs. one third to one fourth in our series). The discrepancies suggest that at least some of the specimens assigned to *O. festae* by Jungfer (2010) may belong to a different species.

Discussion

The phylogenetic relationships recovered by this study are consistent with the phylogenies reported by Wiens et al. (2010) and Moen and Wiens (2009) in finding strong support for a clade that closely allies *O. buckleyi*, *O. verruciger*, and *O. mutabor*. Our clade (*O. mutabor* (*O. festae* (*O. buckleyi-O. verruciger*))) is composed by species that mainly reproduce along streams or slow flowing ditches. At several sites we have found tadpoles of *O. verruciger* in ponds on stream banks and also on slow flowing ditches confirming the riparian habits reported by Trueb and Duellman (1970). Osteocephalus *buckleyi* breed along streams (Jungfer 2010; Lima et al. 2006) and *O. mutabor* has been found breeding along ditches (SRR pers. obs.; Jungfer and Hödl 2002) and temporary ponds (M. Read, pers. comm.) The predominance of stream breeding habits among these species suggests that reproductive mode may be phylogenetically conserved in *Osteocephalus*. The same pattern is suggested by the close relationship between *O. planiceps*, *O. deridens*, *O. fuscifacies* implied by our phylogeny and that of Moravec et al. (2009) because these species share phytotelmata breeding (SRR pers. obs.; Jungfer et al. 2000). This reproductive mode, however, may have an additional independent origin in *O. oophagus* (Moravec et al. 2009).

An unexpected result in our phylogeny is the finding of paraphyly among populations of O. buckleyi and O. verruciger from Ecuador. Plausible explanations include incomplete lineage sorting, mitochondrial gene capture, and the existence of cryptic species hidden within each taxa. In the case of O. verruciger, the position in the phylogeny of the population that generates paraphyly, Pacto Sumaco, is weakly supported (Fig. 2) and the genetic distance between Pacto Sumaco and the other O. verruciger populations is lower (1.5-1.8%) sequence divergence) than the distances between O. *verruciger* and *O. buckleyi* or between any other species pair in the phylogeny (> 2.1%). In addition, we could not find conspicuous morphological differences between Pacto Sumaco and the other O. verruciger populations. The observed pattern suggest that our mitochondrial gene tree may not correctly reflect the history of divergence among O. verruciger and the morphologically distinctive populations of O. buckleyi from Hola Vida and Bobonaza. Conversely, the paraphyly among populations of O. buckleyi has a strong support with higher genetic distances between both clades (4.7%-6.0%). Neither of these clades represent O. cabrerai, a species morphologically similar to O. buckleyi for which there are not confirmed records from Ecuador (Jungfer 2010). Examination of additional characters (morphological and molecular) is underway by SRR to determine the taxonomic status of the populations of O. buckleyi from western Amazonia.

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Appendix I

- Osteocephalus buckleyi.— ECUADOR: PROVINCIA MORONA SANTIAGO: Bobonaza, 660 m (QCAZ 32506–08); PROVINCIA NAPO: Cando, 700 m (QCAZ 24446–47); Ahuano, 410 m (QCAZ 36703); Serena, Río Jatunyacu, 520 m (QCAZ 25321); PROVINCIA ORELLANA: Río Yasuní (QCAZ 7360); Puente del Río Beque, 228 m (QCAZ 43071); Río Rumiyacu, Parque Nacional Yasuní, 250 m (QCAZ 16007); Taracoa, 251 m (QCAZ 34963); Aguarico, Parque Nacional Yasuní (EPN-H 2786); PROVINCIA PASTAZA: Pomona, Fundación Hola Vida, 846 m (QCAZ 25469, 25607, 37175); Nuevo Corrientes, 250 m (QCAZ 14947); Tarangaro, 338 m (QCAZ 39073); Arajuno, Curaray, Río Maderoyacu (EPN-H 6374); PROVINCIA SUCUMBÍOS: Tarapoa (QCAZ 14948); Puerto Bolívar, 240 m (QCAZ 28231); Playas de Cuyabeno, 230 m (QCAZ 28277, 28280, 28395); intersection of the Tarapoa-Puerto Carmen road and Río Cuyabeno, 290 m (QCAZ 28427); PROVINCIA ZAMORA CHINCHIPE: Centro Shuar Yawi, 940 m (QCAZ 31016, 31032–3, 31047, 31051, 31053).
- Osteocephalus deridens.—ECUADOR: PROVINCIA ORELLANA: Estación Científica Yasuní, Universidad Católica del Ecuador, Parque Nacional Yasuní (QCAZ 12556).
- Osteocephalus festae.—ECUADOR: PROVINCIA LOJA: San Francisco, Arco Iris Reserve, Parque Nacional Podocarpus (3.98845° S, 79.09298° W), 2200 m (QCAZ 39364); PROVINCIA MORONA SANTIAGO: Río Napinaza, 6.6 km N from General Leonidas Plaza (Limón) in the road to Mendez (2.92665° S, 78.40701° W), 1010 m (QCAZ 26283, 26304, 26488, 26552, 26561, 32835, 38081, 38420, 39799, 39804-6, 39798-803, 39808-12); San Carlos, San Miguel and Río Oro, 600-1200 m (QCAZ 11624-26); PROVINCIA ZAMORA CHINCHIPE: Miasí Alto (4.25025° S, 78.61740° W), 1250–1300 m (QCAZ 41039); Reserva Tapichalaca (4.55004° S, 79.12914° W), 1637 m (QCAZ 45674); PERU: REGIÓN DE AMAZONAS: PROVINCIA DE BAGUA: Cataratas de Paraiso-Chonza Alta (5.60264 S, 78.3985 W), 1342 m (CORBIDI 760, 761, 762, 763, 764, 758, 759); Camñopite (5.61469 S, 78.33192 W), 1650 m (CORBIDI 1962, 1963, 1964, 1965, 2992); REGIÓN DE SAN MARTÍN: PROVINCIA MARISCAL CACERES: Río Lejia (6.83655 S, 77.48603 W), 1500 m (CORBIDI 623, 624); PROVINCIA RIOJA: Bajo Naranjillo (5.81571, 77.33668 W), 844 m (COR-BIDI 3386).
- Osteocephalus fuscifacies.—ECUADOR: PROVINCIA NAPO: El Tena-Talag Road, 15 km from Tena, 550 m (QCAZ 8806); PROVINCIA ORELLANA: Pompeya-Iro Road, 38 km SE from Pompeya (QCAZ 8137); Estación Científica Yasuní, Universidad Católica del Ecuador, 240 m (QCAZ 20785).

- Osteocephalus mutabor.—ECUADOR: PROVINCIA NAPO: Chontapuntas, Comunidad Sumak Sacha-Pozo Yuralpa Centro 1 (QCAZ 28646–48); Huino, around the waterfall (QCAZ 30916–17, 30919–20, 30922–23, 30925–26); PROVIN-CIA ORELLANA: km 22 Pompeya-Iro Road, 287 m (QCAZ 42999); PROVIN-CIA PASTAZA: Pomona, Fundación Hola Vida, 846 m (QCAZ 25603); Cantón Santa Clara, Río Pucayacu, Colonia Mariscal Sucre (QCAZ 36935, 36946, 40253); PROVINCIA SUCUMBIOS: Puerto Bolívar, 240 m (QCAZ 28223).
- Osteocephalus planiceps.—ECUADOR: PROVINCIA DE NAPO: Chontapuntas, Comunidad Sumak Sacha-Pozo Yuralpa Centro 1 (QCAZ 28648); PROVINCIA ORELLANA: Parque Nacional Yasuní, km 38 Pompeya-Iro Road, 280 m (QCAZ 5134, 14842); Estación Científica Yasuní, Universidad Católica del Ecuador, 240 m (QCAZ 14844, 20797–800); PROVINCIA SUCUMBIOS: La Selva lodge, 250 m (QCAZ 7408, 12093–95).
- Osteocephalus taurinus.—ECUADOR: PROVINCIA ORELLANA: Parque Nacional Yasuní, km 97 Pompeya-Iro road, 450 m (QCAZ 5301); Estación Científica Yasuní, Universidad Católica del Ecuador, 220 m (QCAZ 9007, 10604, 14804, 14954, 24449–50); PROVINCIA SUCUMBÍOS: Reserva de Producción Faunística Cuyabeno, 220 m (QCAZ 5871–77); Puerto Bolívar, 240 m (QCAZ 27916, 27920); Zábalo, 220 m (QCAZ 27982, 28015); Chiritza-Puerto El Carmen road, intersection with Río Aguas Negras, 270 m (QCAZ 28485); Tarapoa-Puerto El Carmen road, intersection with Río Cuyabeno, 290 m (QCAZ 28435–36); PRO-VINCIA ZAMORA CHINCHIPE: Shaime, Nangaritza, 980 m (QCAZ 18230).
- Osteocephalus verruciger.—ECUADOR: PROVINCIA MORONA SANTIAGO: Nueve de Octubre (QCAZ 32266); Bosque Protector Abanico, Morona (EPN-H 11444–45); Río Sardinayacu, Palora, Parque Nacional Sangay (EPN-H 5940–42, 5947); PROVINCIA NAPO: E of Volcán Sumaco, 1570 m (QCAZ 1560, 1562); Lago Agrio road between Cascabel 1 and 2, 1600 m (QCAZ 7783–84); Sumaco, 1800–2100 m (QCAZ 8964); Pacto Sumaco (QCAZ 10907); km 13 (Loreto-Coca road), 1324 m (QCAZ 22201); Río Hollín (QCAZ 1681, 2405); Coordillera de los Guacamayos, Cosanga-Archidona road, 1600 m (QCAZ 12206, 41108); El Reventador (QCAZ 29208); Cascada San Rafael, 1553 m (QCAZ 363, 13225,13247, 16954, 32032–36); Cosanga, 339 m (QCAZ 15942); PRO-VINCIA SUCUMBIOS: Quito-Lago Agrio road, Río Azuela, 1680 m (QCAZ 15149, 15991–97, 16220, 16953, 22497, EPN-H 6341, 11987, 12105–07, 12112, 12143); La Bonita (QCAZ 3175); trail to Volcán Reventador, Gonzalo Pizarro (EPN-H 7052–53, 7059–60).
- Osteocephalus yasuni.—ECUADOR: PROVINCIA SUCUMBIOS: Zábalo, 220 m (QCAZ 27998); Playas de Cuyabeno, 230 m (QCAZ 27816).