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Taxonomy and distribution pattern of the African rain forest butterfly genus *Euphaedra* Hübner *sensu stricto* with the description of three new subspecies of *Euphaedra cyparissa* (Cramer) and one of *E. sarcoptera* (Butler) (Lepidoptera, Nymphalidae, Limenitidinae, Adoliadini)

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Abstract

Updated data on the distribution, ecology and taxonomy of *Euphaedra cyparissa* (Cramer) and *Euphaedra sarcoptera* (Butler) are presented. Three new subspecies of *E. cyparissa* and one of *E. sarcoptera* are described and their geographic distribution is presented. The monophyly of the genus *Euphaedra sensu* Hecq is assessed based on morphological, in particular male and female genitalia, and behavioural traits. Possible evolutionary reasons for the convergence of colour pattern between the sympatric subspecies of *E. cyparissa* and *E. sarcoptera* are discussed.

Keywords

Afrotropical region, colour patterns convergence, *Euphaedra cyparissa*, *Euphaedra sarcoptera*, male and female genitalia, rain forests, subspecies, taxonomy

Introduction

Recent years have witnessed a rising interest in advanced studies focusing on species-rich African brush-footed butterflies (Nymphalidae). Examples include ongoing studies, combining molecular, ecological and morphological data, on the genus *Cymothoe* Hübner (Van Velzen et al. 2007, 2009, 2013) and *Bicyclus* Kirby used as one model group for modern research on genetics and evolutionary ecology (Monteiro and Pierce 2001; Conceição et al. 2011). From this perspective, one of the most speciose African butterfly genera, *Euphaedra* Hübner with nearly 200 recognized species, remains unexplored. This is due to the taxonomic complexity, but most of all to the unstable species-level systematics involved. The latter is a result of the tradition of basing *Euphaedra* taxonomy on simple descriptions of wing colour patterns. It is striking that descriptions of new species of *Euphaedra* have not usually been accompanied by in-depth morphological analysis, nor by ecological (with the honourable exception of Amiet 2004) or biogeographic support, not to mention genetic data (Hecq 1984, 1997, 1999, 2012; Faravel 2002). The morphology of female genitalia of *Euphaedra* remained until recently nearly completely unexplored and was not used with any alpha-taxonomy or in assessing the phylogenetic relations within the genus (Pyrcz et al. 2011). One of the major problems when dealing with species-level *Euphaedra* taxonomy is the huge variation in individual colour pattern evident within some species, on occasion more important to the naked eye than intraspecific differences, compounded by apparently rather frequent intraspecific hybridization producing viable individuals. This, coupled with wide geographic ranges, apparent pattern convergence between sympatric species and the lack of strong, quantitative characters, often impedes reliable recognition of species, which has led to the description of numerous species which are in fact merely individual variations of polymorphic species, or possibly natural hybrids (Hecq 2012). The above mentioned systematic problems effectively hampered any comprehensive studies within this group according to modern evolutionary biology approach, for example using molecular data (Wahlberg et al., in prep.). At this stage, the genus requires more detailed studies at the lower taxonomic level, such as species groups or subgenera, to help prepare the ground for a more broad based revision.

The subgenus *Euphaedra* was delimited by Hecq (1976) in an attempt to break down the extremely diverse genus *Euphaedra sensu* Hübner into several related, possibly monophyletic groups designated as subgenera. His work relied strongly on colour patterns, such as forewing subapical bands and hindwing underside elements as valid characters, and some characters of male genitalia, especially the configuration of cornuti on the vesica and shape of the apical part of valva. According to Hecq (1976) the subgenus *Euphaedra* is identified by the elongated forewing apex and the absence of red scaling on the hindwing underside. Subsequent authors, to a different degree, recognized the entities raised by Hecq, among others, D'Abrera (2004), Larsen (2005) and Vande Weghe (2010). The two species making up the subgenus *Euphaedra sensu* Hecq, *E. cyparissa* (Cramer 1775) and *E. sarcoptera* (Butler 1871), present some apparent morphological and, indeed, behavioural characters that set them apart from other congeners. Schultze (1920) pointed out their less developed, slender thorax. A striking colour pattern element - the pinkish ventral basal patch - present on the forewing in *E. sarcoptera* and absent in *E. cyparissa* makes the immediate separation between the two species unambiguous (Larsen 2005). They are sympatric through most of their range in West and Central Africa. However, *E. sarcoptera* occurs as far east as western Tanzania, whereas *E. cyparissa* does not extend into the Congo basin. Both species are widely polytypic. Distribution of the three subspecies of *E. cyparissa* described so far is puzzling, being either widely disjunct and/or without any apparent geographical pattern. Larsen (2005) discusses three or possibly four subspecies of *E. cyparissa* but in the headings of the species he highlights only two, the nominate and ssp. *tai* Hecq. In the accompanying text, however, he also mentions *aurata* Carpenter and suggests that the Central African Republic population possibly represents a separate subspecies. Vande Weghe (2010) simply illustrates all green specimen, presumably from Gabon, and basically quotes Larsen (op. cit).

Material and methods

Adults of *Euphaedra* were collected using entomological nets and fruit-baited traps. Type specimens deposited in major collections were examined. Male and female genitalia were dissected and compared. Standard dissection protocols were applied, consisting in soaking the abdomens in a warm 10% KOH solution for 35 min., cleaning out of scales and internal organs in distilled water, and staining genital organs with chlorazole black. Genital preparations were placed in glycerol microvials, and pinned under their respective specimens. Photographs of adults were made with an Olympus E-500 digital camera and microscopic structures were photographed under an Olympus stereomicroscope SZX9 equipped with a Nikon Digital sight Ds-Fi1 camera. Plates were composed with Adobe Photoshop 9. Abbreviations used: FW: forewing; HW: hindwing; D: dorsum; V: venter; TL: Type locality.

Collections consulted

ABRI	African Butterfly Research Institute, Nairobi, Kenya
BMNH	The Natural History Museum (formerly British Museum of Natural History), London, UK
DK	Dieuwko Knoop, Tel Aviv, Israel and Boyl, The Netherlands
GF	Gilles Faravel, Pradons, France
HWG	Haydon Warren-Gash, Pressac, France
MIIZ	Muzeum i Instytut Zoologii Polskiej Akademii Nauk, Warszawa, Poland
MRAC	Musée Royal de l'Afrique Centrale, Tervuren, Belgium
MZUJ	Muzeum Zoologiczne Uniwersytetu Jagiellońskiego, Kraków, Poland
RW	Robert Warren (currently in DK)

SZS	Szabolcs Sáfián, Sopron, Hungary
SMTD	Senckenberg Naturhistorische Sammlungen Dresden Museum für Tierkunde
PhO	Philippe Oremans, Montigny-le-Tilleul, Belgique
TL	Torben Larsen (data base)
TWP	Tomasz Wilhelm Pyrcz (to be deposited in MZUJ)

Results

Taxonomic accounts

Euphaedra cyparissa cyparissa **(Cramer)**
http://species-id.net/wiki/Euphaedra_cyparissa_cyparissa
Figs 1A, 1B, 3A, 3B, 9A

Papilio cyparissa Cramer, [1775], *in* Cramer, [1775–1776]: 63, pl. 39. Figs D, E.
Papilio cato Fabricius, 1787: 12 (unnecessary replacement name)

Type-locality. Sierra Leone

Material examined. 1 ♂: Sierra Leone, Guma, 01.03.1982, prep. genit. 10/09.05.2012, J. Lorenc; 1 ♀: same data, prep. genit. 11/09.05.2012, J. Lorenc; 1 ♀: Sierra Leone, Guma Valley, 400 m, 11.1991; 1 ♀: Sierra Leone, no locality, no date; 1 ♂: Ivory Coast, Abidjan (erroneous locality), 1966, S. Collins leg.; 1 ♀: same data (erroneous locality), ABRI; 6 ♂ and 2 ♀: Sierra Leone, Guma, 01.III.1982, H. Warren-Gash leg.; 1 ♂: Guinea, Diecke, 07.2000, HWG; 1 ♂ and 5 ♀: Sierra Leone, SMTD; 4 ♂: Guinée, Guinée forestière, Province de Yomou, Forêt classée de Diecke, 1-26. III.2003, leg. Ph. Leonard and E. Vingerhoedt; 3 ♂ and 1 ♀: same data but VI.2003; 2 ♂: same data but III.2005, PhO; 3 ♂ and 1 ♀: Guinée, Forêt classée de Diecké, GF; 2 ♂ and 2 ♀: Liberia, Mount Swa, Sz. Sáfián leg, MZUJ ; 1 ♂: same data, ABRI.

Diagnosis. Upperside colour black and apple green with a delicate yellow sheen, somewhat more prominent on the HWD median area.

Redescription. Male (Figs 1A, 1B). Head: eyes lustrous, chocolate brown, naked; vertex black with a tuft of short, black hair; labial palpi covered with dense, short, bright yellow hairy scales; antennae half the length of costa, slender, gradually widening into a flattened club, in its widest part only twice as wide as shaft, dorsally black, ventrally bright yellow, covered with sparse sales along most of its length except for terminal segments. Thorax: black, dorsally sparsely covered with black and brown hair, denser laterally, with some violet blue scales on patagium and mesoscutum; tegulae covered with black, grey and brown hair; some longer and denser grey hair on metascutum; legs femora covered with black, tibiae and tarsi with sandy yellow scales. Abdomen: totally covered with dense, black scales, and some bluish scales on first, second and third segments dorsally (apparent only in fresh specimens), and over the entire length laterally. FWD: (length: 31–33 mm, mean: 32, n=9) with an elongated apical part, and gently

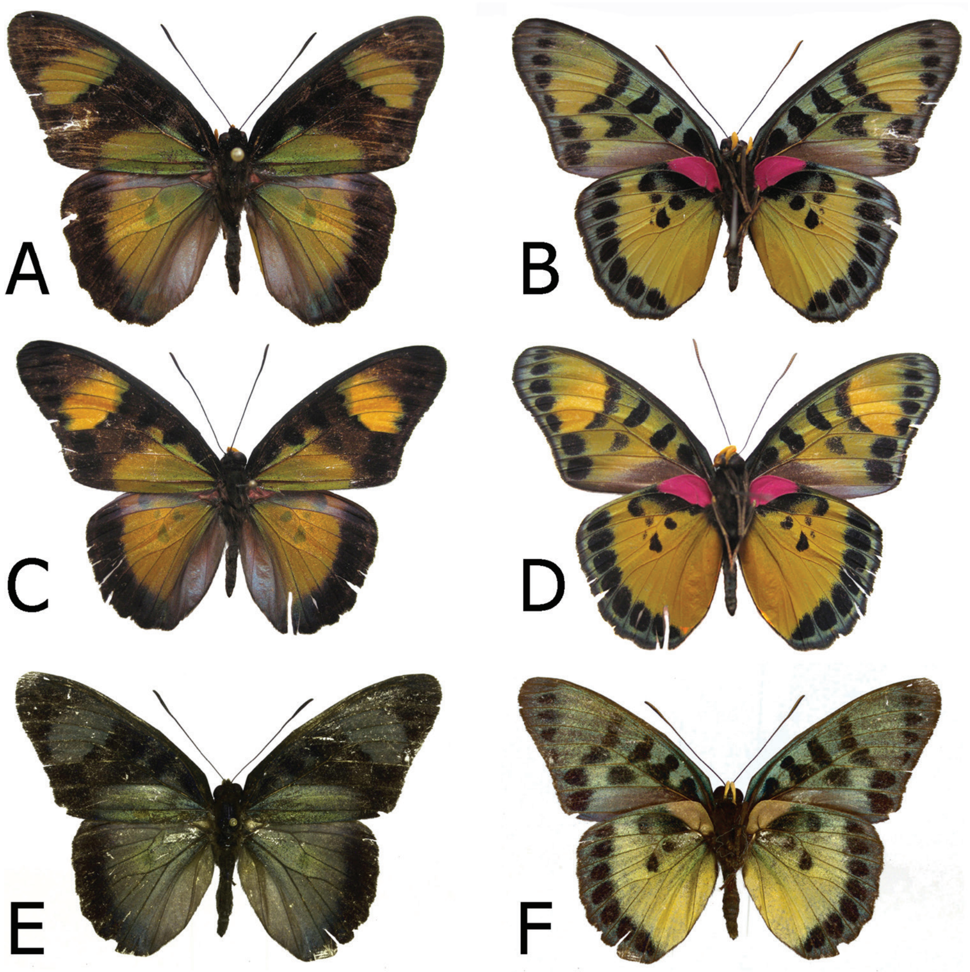

Figure 1. Adults, males: **A** *Euphaedra cyparissa cyparissa* Mount Swa, Liberia (dorsum) **B** *Euphaedra cyparissa cyparissa* Mount Swa, Liberia (venter) **C** *Euphaedra cyparissa nimbina* Mont Nimba, Guinea, holotype (dorsum) **D** *Euphaedra cyparissa nimbina* Mont Nimba, Guinea, holotype (venter) **E** *Euphaedra cyparissa tai* Tai, Ivory Coast (dorsum) **F** *Euphaedra cyparissa tai* Tai, Ivory Coast (venter).

convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a rich green area from wing base to two-thirds of anal margin, marginally entering discal cell along lower part and at base of cell Cu1-Cu; a large, roughly oval rich green subapical patch with a marginal bluish sheen, extending widely from subcosta costa to vein M3, with sharply defined zigzagging basal limit, with an incision along vein M3, and a diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; rich green, with a golden yellow overcast in outer one-third, a black marginal area gradually narrowing from roughly 4–5 mm at apex to 2 mm at tornus where heavily suffused with blue; fringes grey. FWV mostly bottle green, slightly lighter than on the FWD subapical patch, a series of large irregular black discal patches, at wing base, mid cell, cell end, an elongated black patch extending across cells M3-Cu1 and a large, roughly half-moon shaped patch in Cu1-Cu2; a diffused subapical golden yellow patch; a row of seven, roughly oval submarginal patches, two tornal patches in Cu1-Cu2 and Cu2-1/2A irregular and at least twice as big as the remainder. HWV golden green, with an elongated basal pinkish patch extending from costal margin to Rs to one-fourth costa, edged with black; four black median spots, two of which in discal cell, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia* (Fig. 9A). Tegumen one and a half the length of uncus, considerably elongated basally; uncus slender, slightly arched downwards with a sharp tip; gnathos long, one-fourth longer than uncus; pedunculus prominent; saccus flattened in lateral view; valvae with a smooth dorsal surface; aedeagus the length of valva+saccus, tubular and straight, with a sharp distal extremity and prominent cornuti.

Female (Figs 3A, 3B): Sexual dimorphism slight, recognized from the male by the larger size (FW length: 40 mm, n=2). *Female genitalia*. Examined, but damaged and unsuitable for taxonomical use.

Range. The known range of this subspecies extends from Sierra Leone, Liberia to SE Guinea. The pair in ABRI labeled as coming from Abidjan represent a conundrum. However, given the extensive research and collecting by one of the authors (HWG) in the Abidjan area without encountering any other specimens, we have concluded that they must be mislabeled. Larsen (2005) illustrated a male of the nominate subspecies identified as "green form from Ghana", but this particular specimen comes from Sierra Leone (ABRI collection, examined). He also identified another specimen from Ghana as *E. cyparissa cyparissa*, which clearly does not belong to the nominate subspecies but to the new subspecies described below.

Euphaedra cyparissa tai Hecq, 1986

http://species-id.net/wiki/Euphaedra_cyparissa_tai

Figs1E, 1F, 4A, 4B, 9B, 10D

Euphaedra cyparissa taï [sic] Hecq, 1986: 42, figs. 11, 16, 17.

Type-locality. Tai Forest (Forêt de Taï), Ivory Coast

Material examined. 12 ♂: Ivory Coast, Tai, 13–14.05.2000, S. Collins leg.; 1 ♂: same data, prep. genit. 07/09.05.2012, J. Lorenc; 1 ♀: same data; 1 ♀: same data, prep. genit. 08/09.05.2012, J. Lorenc; 1 ♂: same data but XII.1998, K. Adams leg., ABRI; 2 ♂: Ivory Coast , Tai, 12.I.1999, H. Warren-Gash leg.; 1 ♂: Ivory Coast , Tai, 13.I.1999, H. Warren-Gash leg.; 1 ♂ and 1 ♀: Ivory Coast , Tai, XII.1998, K. Adams; 2 ♂ and 1 ♀: Ivory Coast, Tai, 02.I.2000, H. Warren-Gash leg.; 1 ♂ and 1 ♀: Ivory Coast, Tai, 13.V.2000, H. Warren-Gash leg., HWG.

Diagnosis. Upperside colour black and pine green.

Redescription. Male (Figs 1E, 1F): Head, thorax and abdomen: identical to other subspecies. FWD: length 35–37 mm, mean: 36 mm, n=19 with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a bottle green area from wing base to tornus, marginally entering discal cell; a large, roughly rectangular bottle green subapical patch with a bluish sheen, extending widely from subcostal to vein Cu1, with sharply defined zigzagging basal limit, without any incision along vein M3, and somewhat diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; bottle green, gradually turning bluish green from vein M3 towards tornus and anal margin, with a black marginal area with a strong dark blue flush, gradually narrowing from roughly 4–5 mm at apex to 1 mm at tornus; fringes grey. FWV mostly bottle green, slightly lighter than on the upperside, a series of black spots, at wing base, mid cell, cell end, the latter two elongated, a half-moon one in mid cell Cu1-Cu2; two black elongated patches and four spots, two enclosing basally and distally a bottle green patch, and two large, roughly half-moon tornal patches in Cu1-Cu2 and Cu2-1/2A; HWV green with a strong yellow overcast gradually more prominent towards anal margin and tornus, with an elongated basal pinkish patch extending from costal margin to Rs to one-fourth costa, edged with black; two black discal spots, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia* (Fig. 9B): Not differing noticeably from the nominotypical, except for a small, sharp apical tip on the valvae.

Female (Figs 4A, 4B): Sexual dimorphism slight, recognized from the male by the larger size (FW length: 43 mm, n=3). *Female genitalia* (Fig. 10D): Bursa copulatrix very large, roughly oval; no signa; ductus bursae (0.10 mm) approximately the same width over the whole length, half the length of bursa; antrum, here referred as to colliculum (Razowski 1996), slat like with gently folded edges, strongly sclerotized, one-third the length of ductus bursae, shorter than in other subspecies; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis slat-like; papillae anales three times as long (0.14 mm) as wide in lateral view, compressed in ventral view; apophyse posteriores as long as the width of papillae anales; von Siebold organ prominent (but smaller than in some species of the *E. ceres* group (Pyrcz et al. 2011).

Range. The known distribution is limited to the Tai National Park in western Ivory Coast. The same pine green colour can be found in *E. sarcoptera styx* (see below) and also in the local population of *E. judith* Weymer, the latter probably deserving a separate subspecific status (Pyrcz et al. in prep.).

Euphaedra cyparissa nimbina Pyrcz & Warren-Gash, ssp. n.

http://species-id.net/wiki/Euphaedra_cyparissa_nimbina
Figs 1C, 1D, 3C, 3D, 9C, 10C

Type-locality. Mount Nimba, Guinea

Material examined. Holotype ♂: Guinea, Mont Nimba, III.1996, ABRI; Paratypes: (21 ♂ and 9 ♀): 1 ♂: Guinea, Mt. Nimba; 1 ♂: same data; 1 ♀: same data, prep. genit. 09/09.05.2012, J. Lorenc; 1 ♂: Ivory Coast, Mont Nimba, 01.1998, S. Collins leg., ABRI; 2 ♂: Guinée, Nimba, I.1998; 2 ♂ and 1 ♀: Guinée, Nimba, VI.1998; 1 ♀: Guinée, Nimba, X.1998; 1 ♂ and 1 ♀: Guinée, Labé, Fouta Djalon, X.1998; 1 ♂: Guinea, Sérédou, VI.1999; 5 ♂ and 1 ♀: Guinea, Mont Nimba, VI.2000, HWG; 1 ♂: Guinée, Mont Nimba, 02.V.1998, ex HWG; 1 ♂: same data but VI.2000; 1 ♀: same data; MZUJ; 7 ♂: Guinée, Mt. Nimba, VII.2004, E. Vingerhoedt leg.; 3 ♀: same data, PhO.

Diagnosis: Upperside black and golden green, similar to *E. sarcoptera ferrea* ssp. n., little difference in shade between basal area and subapical patch, except for its slightly more prominent yellow shade.

Description. Male (Figs 1C, 1D): Head, thorax and abdomen: identical to other subspecies. FWD: (length: 34–42 mm, mean: 38.5 mm, n=16) with an elongated apical part and gently convex outer margin; most of wing surface black; a golden green area from wing base to two-thirds of anal margin, covering lower one-third of discal cell, enclosing a mid discal cell black spot; a large, roughly rectangular golden yellow subapical patch with a greenish overcast along inner and outer margin, extending widely from subcosta to vein Cu1, with sharply defined zigzagging basal limit and somewhat diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; uniform golden green, a black marginal area with a dark blue flush, roughly 4–5 mm wide with smooth and sharply defined basal edge (compared to more diffuse and zigzagging in the nominate); fringes grey. FWV mostly golden green, a series of black spots, at wing base, mid cell, cell end, the latter two elongated, a half-moon one in mid cell Cu1-Cu2; two black elongated patches and four spots, two enclosing basally and distally a rich yellow patch, and two large, roughly oval tornal patches in Cu1-Cu2 and Cu2-1/2A. HWV golden green with, with an elongated basal pinkish patch extending from costal margin to Rs to one-fourth costa, edged with black; two black discal spots, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia* (Fig. 9C): Does not differ noticeably from the nominotypical, except for a small, sharp apical tip on the valvae.

Female (Figs 3C, 3D): Sexual dimorphism slight, recognized from the male by the larger size (43–50 mm, mean: 46 mm, n=5). *Female genitalia* (Fig. 10C): Bursa copulatrix large, oval; no signa; ductus bursae (0.19 mm) narrow, approximately the same width over the whole length, two-thirds the length of bursa; colliculum wide, slat like with folded edges, strongly sclerotized, one-third the length of ductus bursae; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis small, slat-like; papillae anales three times as long (0.14 mm) as wide in lateral view, compressed in ventral view; apophyse posteriores as long as the width of papillae anales; von Siebold organ prominent (but smaller than in some species of the *E. ceres* group (Pyrcz et al. 2011).

Etymology. The subspecific epithet of this taxon derives from its type locality, the massif of Mount Nimba (1750 m) on the Ivory Coast – Guinea border.

Range. The range is apparently disjunct with most specimens coming from the lower slopes of Mont Nimba and the surrounding area, and two collected in the Fouta Djalon range in northern Guinea, the latter record needs confirmation.

Euphaedra cyparissa aurantina Pyrcz & Oremans, ssp. n.

http://species-id.net/wiki/Euphaedra_cyparissa_aurantina
Figs 2A, 2B, 3E, 3F, 9D, 10A, 10B

Type-locality. N. Awasso, Bibiani, Western Region, Ghana

Material examined. Holotype ♂: Ghana, Western, Bibiani, N. Awasso, 250–400 m, 02.IV.2007, MZUJ; Paratypes (127 ♂ and 29 ♀): 7 ♂: Ivory Coast, Abengourou, XI.1993, S. Collins leg., (1 ♂: prep. genit. 03/09.05.2012, J. Lorenc); 1 ♂: Ivory Coast, Abengourou, 13.V.1973 ; 1 ♀: Ivory Coast, Abengourou, 04.XII.1970, J. Kielland leg., prep. genit. 03/09.05.2012, J. Lorenc; 1 ♀: Ivory Coast, Abengourou; 1 ♀: Ivory Coast, Abengourou, S. Collins leg.; 1 ♂: Ivory Coast, Danane, IV.1973, S. Collins leg.; 1 ♀: same data but IV.1977; 1 ♀: same data but IV.1970 (all 3 specimens from « Danane » possibly mislabeled) ; 3 ♂: Ghana, Ankasa, XI.2003, Vorgas/Yevu leg.; 1 ♀: Ghana, Bia, XII.2003, Vorgas/Yevu leg.; 1 ♀: same data, prep. genit. 06/09.05.2012, J. Lorenc; 13 ♂: Ghana, Bia, XII.2003, Vorgas/Yevu leg.; 7 ♂: Ghana, Bia, III.2003; 1 ♀: Ghana, Bibiani, IV.2007, Vorgas/Yevu leg.; 1 ♂: Ghana, Bibiani, IV.2007, SY leg.; 1 ♀: Ghana, Bonkro, XI.2005, Vorgas/Yevu leg.; 5 ♂: Ghana, Cape 3 Points, I.2004, R. Vorgas leg.; 1 ♀: same data but I.2006, 1 ♂: Ghana, Kibi, XII.1996, S. Collins leg.; 1 ♂: Ghana, Kibi, S. Collins leg.; 1 ♂: Ghana, Mate, II.2002, Vorgas/Yevu leg.; 1 ♂: same data but III.2002; 1 ♂: Ghana, Mpasaso, VII.2004, Vorgas/Yevu leg.; 1 ♀: same data but V.2004, prep. genit. 02/09.05.2012, J. Lorenc; 1 ♂: Ghana, Nkwakwa, 04.IX.2000; 1 ♀: Ghana, Nkwakwa, IV.2000, S. Collins leg.; 3 ♂: Ghana, Sagamase, Kibi, X.2010, S. Collins leg.; 3 ♂: same data but XII.2000; 2 ♂: same data but IX.2003; 2 ♂: same data but I.2002; 1 ♂: same data but I.2001; 3 ♂: same data but XII.1997; 1 ♂: same data but IV.2003; 1 ♂: same data but VII.1998; 1 ♀: same data but I.2002; 1 ♀: same data but I.2003; 11 ♂: Ghana, Tano Ofin, III.2002, Vorgas/Yevu leg.; 1 ♂: same data, prep. genit. 01/09.05.2012, J. Lorenc; 13 ♂: same data but I.2004; 3 ♂: same data but II.2002; 1 ♂: same data but X.2009; 1 ♂: same data but IX.2002; 1 ♂: same data but XI.2003; 1 ♂: same data but IX.2001; 1 ♂: same data but III.2001; 4 ♂: same data but II.2001, R. Vorgas leg.; 1 ♂: same data but Vorgas/Yevu leg.; 1 ♂: same data but S. Collins leg.; 1 ♂: same data but XII.2001, S. Collins leg.; 4 ♂: same data but III.2001; 1 ♂: Ghana, Tano Ofin, I.2011; 1 ♀: same data but IV.2011; 2 ♀: same data but III.2002, Vorgas/Yevu leg.; 1 ♀: same data but II.2002; 1 ♀: same data but II.2001, R. Vorgas leg.; all ex coll. ABRI; 2 ♂: Ghana, Kyebi (Kibi) District, Eastern Region, Segyimaase, forestry access road, Atewa Range, 29.III-04.IV.2005, Sáfián, Sz., Csontos, G. and Kormos, B. leg.; 1 ♂: same data but 13–21.X.2007, Sáfián, Sz. leg., SZS; 1 ♀: Ghana, Eastern Region, Asuom Amanfrom, Amanfrom Forest, Kade District, 20–24.III.2005, Sáfián, Sz., Csontos, G. and Kormos, B. leg., SZS; 1 ♂: Ghana, Western, Bibiani, N. Awasso, 250–400 m, 01.IV.2007; 1 ♀: Ghana, Eastern Region, Atewa Forest, Sagymasse trail, 400–500 m, 16–27.X.2008, G. Csontos and Sz. Sáfián leg., MZUJ; 1 ♀: Ivory Coast, Banco, 11.XII.1999, H. Warren-Gash leg.; 1 ♀: Ivory Coast, South-West, Ft de Dassioko, 31.XII.1997, H. Warren-Gash leg.; 1 ♂: Ivory Coast, Foret d'Azagny,

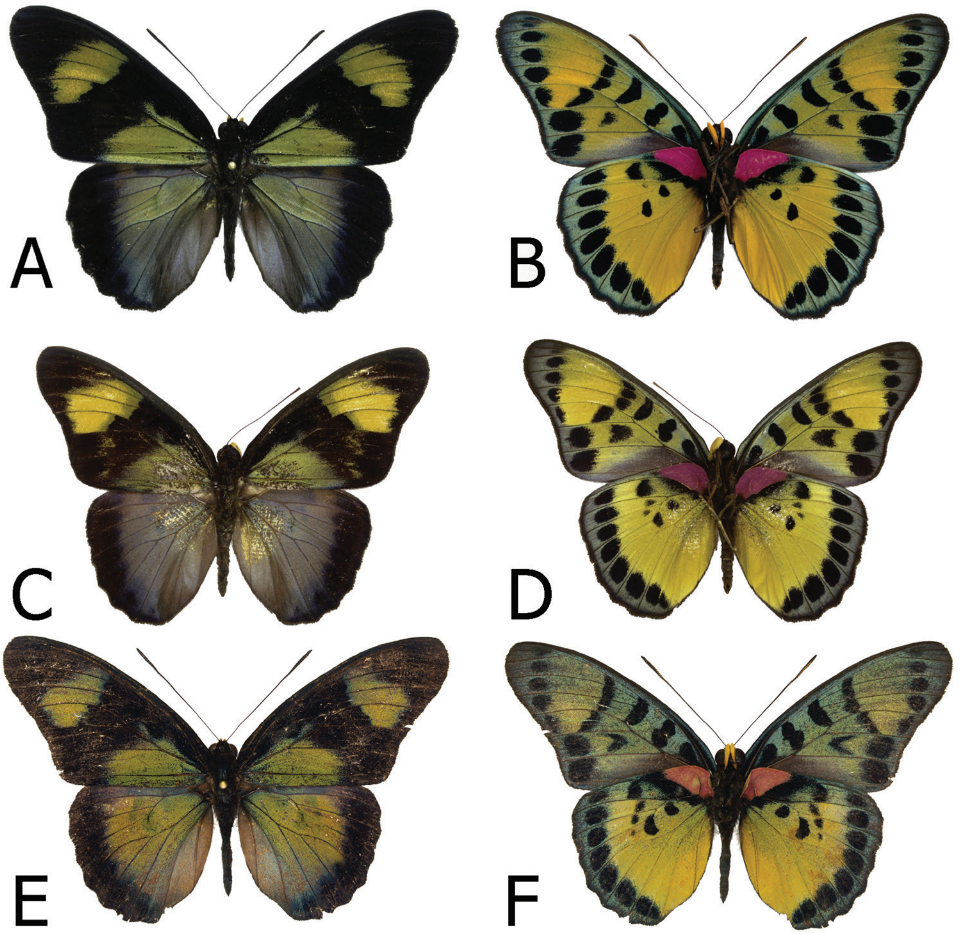

Figure 2. Adults, males: **A** *E. cyparissa aurantina* Bibiani, Ghana, holotype (dorsum) **B** *E. cyparissa aurantina* Bibiani, Ghana, paratype (venter) **C** *E. cyparissa aurata* Isheri, Nigeria (dorsum) **D** *E. cyparissa aurata* Isheri, Nigeria (venter) **E** *E. cyparissa nominalina* Mongoumba, R. C. A., holotype (dorsum) **F** *E. cyparissa nominalina* Mongoumba, R. C. A., holotype (venter).

10.III.2001, H. Warren-Gash leg.; 1 ♂: Ivory Coast, Foret d'Azagny, 06.I.2001, H. Warren-Gash leg., HWG; 14 ♂ and 7 ♀: Kibi, Ghana, XII.2012, local collector leg., Ph.O ; 2 ♂ and 1 ♀: same data, MZUJ.

Diagnosis. Upperside black and golden green with a yellow subapical patch strongly suffused with green, compared to the slightly suffused patch in *Euphaedra cyparissa aurata*.

Description. Male (Figs 2A, 2B): Head, thorax and abdomen: identical to other subspecies. FWD: (length: 34–38 mm, mean: 37 mm, n=4): with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a yellow green area with a golden sheen from wing base to two-thirds of anal margin, slightly entering discal cell and base of cell Cu1-Cu2;

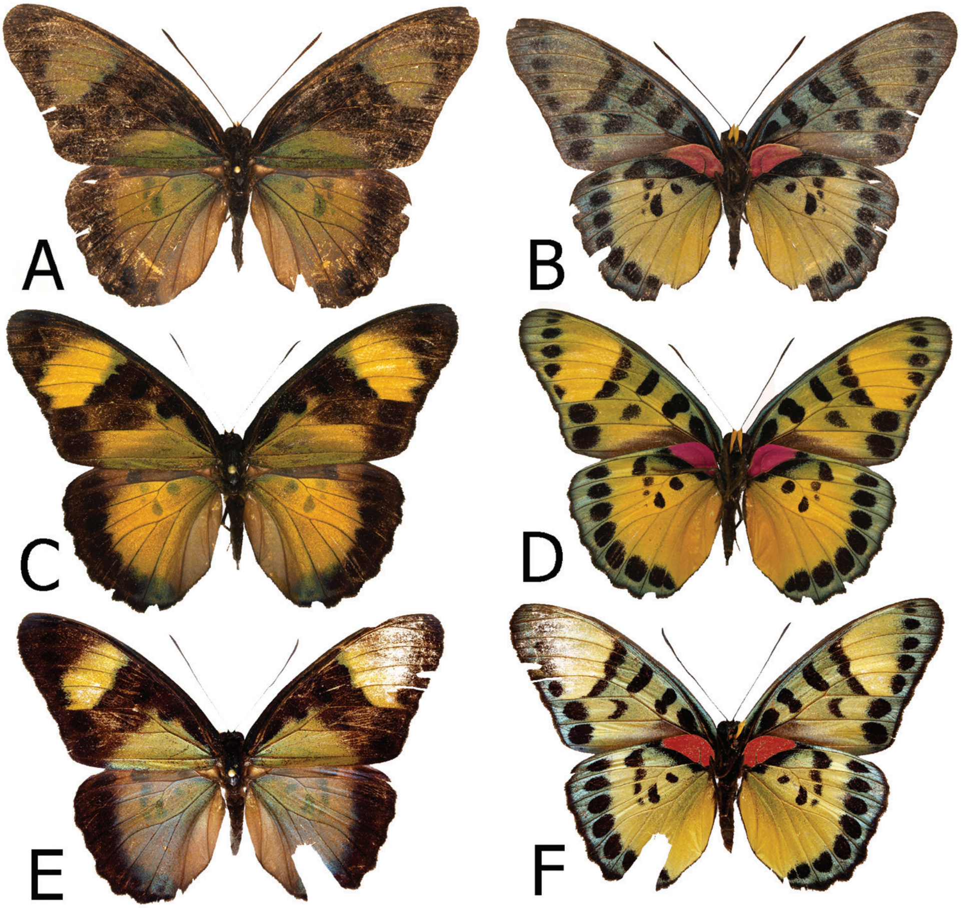

Figure 3. Adults, females: **A** *E. cyparissa cyparissa* Guma, Sierra Leone (dorsum) **B** *E. cyparissa cyparissa* Guma, Sierra Leone (venter) **C** *E. cyparissa nimbina* Mont Nimba, Guinea, paratype (dorsum) **D** *E. cyparissa nimbina* Mont Nimba,Guinea, paratype (venter) **E** *E. cyparissa aurantina* Atewa Forest, paratype (dorsum) **F** *E. cyparissa aurantina* Atewa Forest, paratype (venter).

a large, roughly rectangular yellow subapical patch heavily dusted with green scales, with sharply defined zigzagging basal limit and somewhat diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; predominantly golden green, gradually turning bluish green from vein M3 towards tornus and anal margin, with a blackish marginal area with a strong dark blue flush, gradually narrowing from roughly 4–5 mm at apex to 1 mm at tornus, leaving a dark blue submarginal dot in cell Cu1-Cu2; fringes grey. FWV mostly green yellow except for a large rich yellow subapical patch with some greenish overcast in apical part, a series of black spots, at wing base, mid cell, cell end, the latter two elongated, a half-moon one in mid cell Cu1-Cu2; two black elongated patches and four spots, two enclosing basally and distally a mostly rich yellow patch suffused with green yellow in its apical part, and two large, roughly

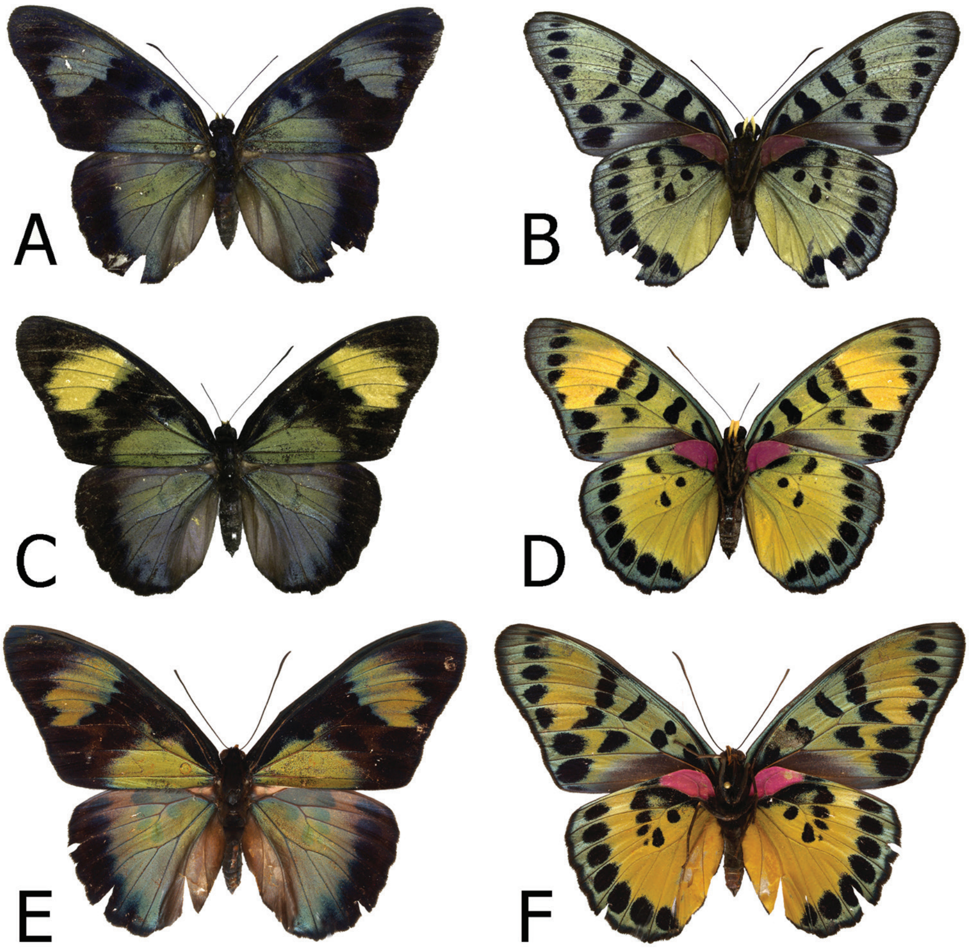

Figure 4. Adults, females: **A** *E. cyparissa tai* Tai, Ivory Coast (venter) **B** *E. cyparissa tai* Tai, Ivory Coast (venter) **C** *E. cyparissa aurata* Isheri, Nigeria (dorsum) **D** *E. cyparissa aurata* Isheri, Nigeria (venter) **E** *E. cyparissa nominalina* Borgen Dolphin Platform, paratype (dorsum) **F** *E. cyparissa nominalina* Borgen Dolphin Platform, paratype (venter).

square tornal patches in Cu1-Cu2 and Cu2-1/2A; HWV mostly rich yellow with an elongated basal pinkish patch extending from costal margin to Rs to one-third costa, edged with black; two black discal spots, and a series of smaller patches forming a gradually shrinking row from mod costa to discal cell end, a row of eight large, mostly rectangular black submarginal patches; marginal area steely blue. *Male genitalia* (Fig. 9D): Not differing noticeably from the nominotypical subspecies.

Female (Figs 3E, 3F): Sexual dimorphism slight, recognized from the male by the larger size (FW length: 43 mm, n=3). *Female genitalia* (Figs 10A, 10B): Bursa copulatrix large, oval; no signa; ductus bursae (0.21–0.24 mm) longer than in other subspecies, narrow, approximately the same width over the whole length, same length as bursa; colliculum slat like with gently folded edges, strongly sclerotized, one-third the length of ductus bursae; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis small, slat-like; papillae anales three times as long (0.9–0.13 mm) as wide in lateral view, compressed in ventral view; apophyse posteriores as long as the width of papillae anales; von Siebold organ prominent (but smaller than in some species of the *E. ceres* group (Pyrcz et al. 2011).

Etymology. The epithet of this taxon is an allusion to its resemblance to the next subspecies, *Euphaedra cyparissa aurata*.

Range. A widely distributed subspecies found in coastal forests in the Ivory Coast and Ghana, and also in inland forests in eastern Ivory Coast and Ghana.

Euphaedra cyparissa aurata Carpenter, 1895

http://species-id.net/wiki/Euphaedra_cyparissa_aurata
Figs 2C, 2D, 4C, 4D, 9E, 10E

Euphaedra cyparissa var. *aurata* Carpenter, 1895: 305.
Euphaedra cyparissa aurata Hecq, 1999: 4, 5

Type-locality. Lokoja, Nigeria

Material examined. 1 ♂: Nigeria, Butatong, Calabar, 14.XI.1995, S. Collins leg.; 1 ♀: same data but 14–18.XI.1995; 2 ♂: Nigeria, east, T. Davey leg.; 1 ♀: same data; 1 ♂: Nigeria, Lagos, IX.1957, J. Boorman leg.; 1 ♀: same data but X.1962; 3 ♂: Nigeria, Mamu Forest, IV.1966, S. Collins leg.; 1 ♀: same data; 8 ♂: Nigeria, Obudu, 09–12.XI.1995, S. Collins leg.; 1 ♂: same data, prep. genit. 12/09.05.2012, J. Lorenc; 2 ♀: same data; 1 ♀: same data, prep. genit. 13/09.05.2012, J. Lorenc; 1 ♂: Nigeria, Okrasa, Calabar, S. Collins leg.; 1 ♀: Nigeria, Old Ekuri Calabar, 24–28.XI.1995, S. Collins leg.; 2 ♀: Nigeria, Olokomeji, IX.1957, J. Boorman leg.; 2 ♂: Cameroon, Ebogo, IV.1983, S. Collins leg., prep. genit. 16/09.05.2012, J. Lorenc, ABRI; 3 ♀: Nigeria, Isheri F., XII.1989, II-III.1990, A. A. Knoop Lindeboom leg; 2 ♂: Nigeria, Oban XI.1995, T. B. Larsen leg.; 1 ♂: Nigeria, Anambra F. R., VII.1962, St. Leger leg.; 6 ♂: Nigeria, Isheri F., IV.1988, II.1989, IV.1989, XII.1989, II.1990, A. A. Knoop Lindeboom leg.; 1 ♂: Nigeria, Isheri F., II.1991, D. P. Knoop leg. DK; 2 ♂: Nigeria, Isheri Forest, 29.III.1989, A. Knoop leg.; 3 ♂: same data but 09.V.1990; 1 ♂: same data but 23.I.1990; 1 ♂: same data but 14.II.1990; 1 ♂: same data but 09.IV.1990; 2 ♀: same data but I.1990; 1 ♀: same data but 10.III.1990; 1 ♀: same data but 05.IV.1989; 1 ♀: same data but 18.II.1990; 1 ♂: Nigeria, Olokomeji, 16.V.1988, A. Knoop leg.; 1 ♂: same data but 20.IV.1988; 1 ♀: same data; 1♂: Nigeria, Anambra State, Nsukka, 28.IX.1982, J. Wojtusiak leg.; 1 ♂: same data but 01.II.1983; 2 ♂: no data, MZUJ; 1 ♂: Nigeria, Olokomeji, 13.V.1989, leg. A. Knoop; 1 ♀: same locality, 20.IV.1988, leg. A. Knoop, PhO; 1 ♀: Gabon, Mondah, VIII.1993, leg. G. Faravel, GF.

Diagnosis. Upperside colour black and mint green with a golden yellow subapical patch, usually with little or no green overcast.

Redescription. Male (Figs 2C, 2D): Head, thorax and abdomen: identical to other subspecies. FW length 29–41 mm, mean: 36 mm, n=42 (one exceptionally small specimen, 25 mm, was examined). FWD: with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a yellow green area from wing base to two-thirds of anal margin, slightly entering discal cell and base of cell Cu1-Cu2; a large, roughly rectangular rich yellow subapical patch dusted along the edges with yellow green scales, with sharply defined zigzagging basal limit and somewhat diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; predominantly steely bluish with some green yellow scaling in discal cell and in cells M1-M2 and M2-M3, with a blackish marginal area with a strong dark blue flush, gradually narrowing from roughly 4–5 mm at apex to 1 mm at tornus, leaving a dark blue submarginal dot in cell Cu1-Cu2; fringes grey. FWV mostly green yellow except for a large rich yellow subapical patch with some greenish overcast in apical part, a series of black spots, at wing base, mid cell, cell end, the latter two elongated, a half-moon one in mid cell Cu1-Cu2; two black elongated patches and four spots, two enclosing basally and distally a mostly rich yellow patch suffused with green yellow in its apical part, and two large, roughly square tornal patches in Cu1-Cu2 and Cu2-1/2A; HWV mostly rich yellow with an elongated basal pinkish patch extending from costal margin to Rs to one-third costa, edged with black; two black discal spots, and a series of smaller patches forming a gradually shrinking row from mod costa to discal cell end, a row of eight large, mostly rectangular black submarginal patches; marginal area steely blue. *Male genitalia* (Fig. 9E): Not differing noticeably from the nominotypical except for a small, sharp apical tip on the valvae.

Female (Figs 4C, 4D): FW length: 40–48 mm. Sexual dimorphism slight, recognized from the male by the larger size (FW length: 39–47mm, mean: 44mm, n=16). *Female genitalia* (Fig. 10E) Bursa copulatrix large, rounded; no signa; ductus bursae (0.16 mm) narrow, approximately the same width over the whole length, two-thirds the length of bursa; colliculum slat like with gently folded edges, strongly sclerotized, half the length of ductus bursae; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis small, slat-like; papillae anales three times as long (0.11 mm) as wide in lateral view, compressed in ventral view; apophyse posteriores as long as the width of papillae anales; von Siebold organ prominent (but smaller than in some species of the *E. ceres* group (Pyrcz et al. 2011).

Range. Found in the rain forests throughout southern Nigeria, western Cameroon and Gabon. An individual (a female) collected by Faravel in Mondah, Gabon, is tentatively associated with this subspecies although it is lighter orange on the FW and HWD, and is therefore similar to *E. cyparissa nimbina* and also to *E. sarcoptera nipponicorum*, with which it was confused by Vande Weghe (2010). More sampling from southern Cameroon and Gabon is needed to evaluate the subspecific status of this population.

Euphaedra cyparissa nominalina **Pyrcz & Knoop, ssp. n.**

http://species-id.net/wiki/Euphaedra_cyparissa_nominalina
Figs 2E, 2F, 4E, 4F, 9F, 10F

Euphaedra cyparissa [Cramer], Vande Weghe, 2010: 115, fig. 15 (male, dorsum), 304–305.

Type-locality. Mongoumba, Central African Republic

Material examined. Holotype ♂: Central African Republic, Mongoumba, VII.1969, ABRI; Paratypes (5 ♂ and 4 ♀): 1 ♂: Central African Republic, Bangui, VIII.2000, S. Collins, prep. genit. 14/09.05.2012, J. Lorenc; 1 ♂: Central African Republic, near Bangui, 12.II.1969, J. Kielland leg.; 2 ♂: Central African Republic, Bomoloto, R. Loanye, VII.1985, S. Collins leg.; 1 ♀: Central African Republic, Mongoumba, 19.VII.1969, S. Collins leg.; 1 ♀: same data prep. genit. 15/09.05.2012, J. Lorenc; 1 ♀: Central African Republic, no locality, ABRI; 1 ♀: Off Shore Nigeria, Platform Borgen Dolphin, 4°18'98"35 N, 8°22'00"32 E, VIII. 2005, ex coll. P. Kowalski, MZUJ; 1 ♂: Cameroon, SE, Moloundou, 500 m, 25.VII.1989, E. Joly leg., PhO.

Diagnosis. Upperside colour black and olive drab, same as in the nominate *E. cyparissa*, compared to golden green in *E. cyparissa aurata* and *E. cyparissa aurantina*, or pine green in *E. cyparissa tai*; differing from the widely separated geographically nominate *E. cyparissa* only in the slightly wider FWD subapical patch towards costal margin.

Description. Male (Figs 2E, 2F): Head, thorax and abdomen: identical to other subspecies. FWD: (length 32–36 mm, mean: 34.9 mm, n=6) with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a rich green area from wing base to three-fourths of anal margin, entering discal cell along lower part from base to root of vein Cu1, enclosing a black mid cell spot; a large, roughly rectangular bottle green subapical patch with a bluish sheen, extending widely along costa, with sharply defined zigzagging basal limit, without any notable incision along vein M3, and a diffused outer limit; fringes grey. HWD oval with a gently scalloped outer margin; rich green, gradually turning bluish green from vein M3 towards tornus and anal margin gradually narrowing from roughly 4–5 mm at apex to 2 mm at tornus; fringes grey. FWV mostly bottle green, slightly lighter than on the FWD subapical patch, a series of black spots, at wing base, mid cell, cell end, the latter two elongated, extending across cells M3-Cu1 and Cu1-Cu2; a row of seven, roughly oval submarginal patches, two tornal patches in Cu1-Cu2 and Cu2-1/2A twice as big as the remainder. HWV golden green, with an elongated basal pinkish patch extending from costal margin to Rs to one-fourth costa, edged with black; two black discal spots, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia* (Fig. 9F): Not differing noticeably from the nominotypical.

Female (Figs 4E, 4F): Sexual dimorphism slight, recognized from the male by the larger size (FW length 50 mm, n=1). *Female genitalia* (Fig. 10F): Bursa copulatrix large, rounded; no signa; ductus bursae (0.22 mm) narrow, approximately the same width over the whole length, two-thirds the length of bursa; colliculum slat like with gently folded edges, strongly sclerotized, half the length of ductus bursae; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis small, slat-like; papillae anales three times as long (0.12 mm) as wide in lateral view, compressed in ventral view; apophyse posteriores slightly longer than the width of papillae anales; von Siebold organ prominent (but smaller than in some species of the *E. ceres* group (Pyrcz et al. 2011).

Etymology. The epithet of this taxon is an allusion to its resemblance to the nominotypical subspecies.

Range. On present knowledge, the range of this subspecies is limited to the CAR and eastern Cameroon, though its distribution pattern indicates it also occurs in adjacent areas of the DRC. The individual collected on an offshore platform in the Gulf of Guinea is an extraordinary finding. It is also a proof that *E. cyparissa* is prone to long distance dispersal. Other butterfly species collected on the same platform were typical dispersalists, including *Danaus, Acraea* and *Melanitis*. It is nonetheless surprising to find in that area an individual which clearly matches the phenotype of *E. cyparissa nominalina*, whereas the distribution pattern would rather suggest the presence of the subspecies *E. cyparissa aurata* along the Atlantic coast of Cameroon.

***Euphaedra sarcoptera sarcoptera* (Butler, 1871)**

http://species-id.net/wiki/Euphaedra_sarcoptera_sarcoptera
Figs 5A, 5B, 7A, 7B, 11A

Romaleosoma sarcoptera Butler, 1871: 81, pl. 31, fig. 2.
Euphaedra sarcoptera (Butler); Aurivillius, 1925: 186.

Type-locality. Ghana "Gold Coast"

Material examined. 1 ♂: Western Ghana, Bibiani, N. Awaso, 250–400 m, 09.IV.2007, ABRI; 1 ♂: same data, prep. genit. 04/20.06.2012, J. Lorenc; 1 ♀: Nsukka, Anambra State, Nigeria, 04.XII.1982, leg. J. Wojtusiak, prep. genit. 02/20.06.2012, J. Lorenc; 1 ♀: Banco N. P., Ivory Coast, 18.IX.1999, H. Warren-Gash leg., prep. genit. 05/20.06.2012, J. Lorenc, MZUJ; 1 ♂: Ghana, Oda Big Tree, XII.2010, Sáfián, Sz. leg., SZS; 1 ♂: Ghana, Eastern Region, Asuom Amanfrom, Amanfrom Forest, Kade District, 20–24.III.2005, Sáfián, Sz., Csontos, G. and Kormos, B. leg., SZS; 1 ♂: Ivory Coast, Alepe-Yaya, 06.II.1999, H. Warren-Gash leg.; 1 ♂: Ivory Coast, Alepe-Yaya, 12.XII.1999, H. Warren-Gash leg. 1 ♂: Ivory Coast, Lamto, 05.XI.2000, H. Warren-Gash leg.; 2 ♂ and 2 ♀: Ivory Coast, Alepe, 21.I.2000, H. Warren-Gash leg.; 1 ♀: Ivory Coast, Alepe, 07.I.2001, H. Warren-Gash leg., HWG; 1 ♂ and 1 ♀: Ivory Coast, Teapleu, V.1977; 10 ♂ and 3 ♀: Guinée, Guinée forestière, Province de Yomou, Forêt classée de Diecke, VI.2003, Eric Vingerhoedt leg.; 1 ♂: Cameroon, Douala, IX.1996, leg. P. Prouvost; 1 ♂: Cameroon, no exact locality, II.1997, E. Vingerhoedt, 1 ♂: Cameroon, Ebogo, I.1994, T. Garnier leg., PhO; 40 ♂ and 28 ♀: Ghana; 2 ♂ and 2 ♀: Ivory Coast; 1 ♂: S. Nigeria, Ewohimi, 29.XI.55, J. Boorman;

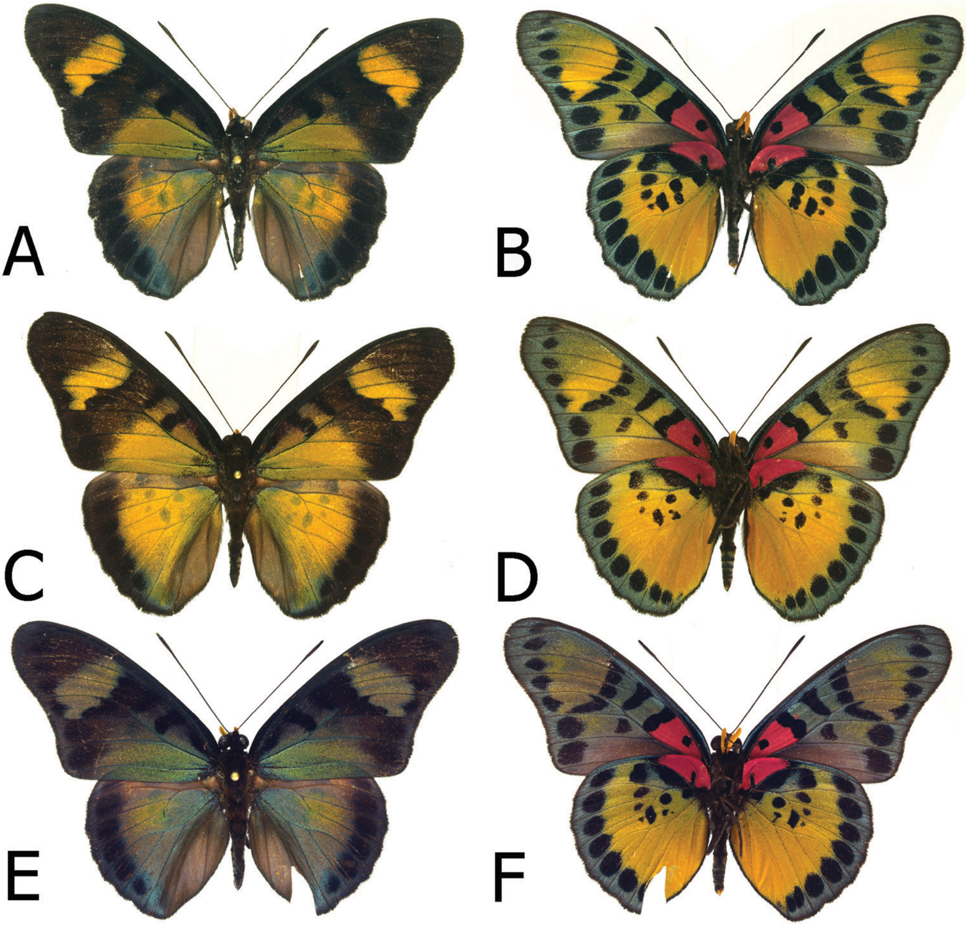

Figure 5. Adults, males: **A** *E. sarcoptera sarcoptera* male, Bibiani, Ghana (dorsum) **B** *E. sarcoptera sarcoptera*, Bibiani, Ghana (venter) **C** *E. sarcoptera ferrea* Mont Nimba, Guinea, holotype (dorsum) **D** *E. sarcoptera ferrea* Mont Nimba, Guinea, holotype (venter) **E** *E. sarcoptera styx*, Tai, Ivory Coast (dosum) **F** *E. sarcoptera styx*, Tai, Ivory Coast (venter).

1 ♂: no label; 2 ♂: Cameroon, Ebogo, Mbalmayo, X.2001; 1 ♂: Cameroon, Ebogo, II.1994, S. Collins leg., ABRI; 1 ♂: Ghana, Kibi Atewa Mts., II.2007., J. Boersma; 1 male ♂ : Nigeria, Ewohimi, I.1955, J. St. Leger leg., DK ; 2 ♂: Nigeria, Sapoba, III,1967, J. Riley leg., ex Cornes and Riley coll., DK; 2 ♂ and 2 ♀: Liberia, Mount Swa, Sz. Sáfián leg, MZUJ; 1 ♂: same data, ABRI; 1 ♂: same data, SSS.

Diagnosis. Upperside ground colour predominantly golden green, with the exception of the light yellow subapical patch.

Redescription. Male (Figs 5A, 5B): Head: eyes lustrous, golden brown, naked; vertex black with a tuft of short, black hair with a bluish sheen; labial palps covered with dense, short, bright yellow hairy scales; antennae half the length of costa, slender, gradually widening into a flattened club, in its widest part only twice as wide as shaft, dorsally and ventrally all black, covered with sparse white and black sales along most of its length except for terminal segments. Thorax: black, dorsally sparsely covered with black and brown hair, denser laterally, with some violet blue scales on patagium and mesoscutum; tegulae covered with black hair; some longer and denser black hair on metascutum; legs femora, tibiae and tarsi with black scales. Abdomen: totally covered with dense, black scales, ventrally with some grey scales. Wings (FW length: 35–39 mm, mean: 37 mm, n=8) with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a golden green area from wing base to two-thirds of anal margin, covering lower half of discal cell; a prominent mid discal cell black round spot; a large, elongated light yellow subapical patch suffused with green, extending widely from subcosta costa to vein Cu1, with a nearly straight basal limit marked by an incision along vein M3, and a diffused outer limit; fringes grey and black. HWD oval with a gently scalloped outer margin; rich golden green, with a diffuse golden postdiscal patch and a strong shiny blue overcast in outer one-third from vein Cu2 to anal margin; submarginal and marginal area black with a strong blue sheen gradually narrowing from roughly 4 mm at apex towards tornus and transforming into rectangular patches, the last of which in Cu2-1-2A detached; fringes grey and black. FWV golden green, slightly lighter than on the FWD; basal to mi-cell area purple; a series of black patches, a rounded postbasal spot, a wide mid cell bar, a similar bar along discal cell distal margin, a series of half-moon shaped patches along basal limit of a diffused subapical light yellow patch, a large half-moon shaped patch in mid cell Cu1-Cu2, and a series of submarginal patches, five smaller from costa to cell M3-Cu1 and two larger, oval, in Cu1-Cu2 and Cu2-1-2A. HWV golden yellow, with an elongated basal purple patch extending from costal margin to Rs to one-fourth costa, edged with black; six black median spots, four of which in discal cell, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia* (Fig. 11A): Tegumen same length as uncus, slightly arched; uncus stout, tip slightly curved downwards; gnathos long, same length as uncus; pedunculus prominent; saccus flattened in lateral view; valvae with a smooth dorsal surface and blunt apex; aedeagus as long as valvae, tubular and straight, with a sharp distal extremity and prominent cornuti.

Female (Figs7A, 7B): Sexual dimorphism slight except for the larger size of the female (FW length: 41–45 mm, mean: 43 mm, n=5) and duller dorsal colours. *Female genitalia*: not examined.

Range. The nominate subspecies is widely distributed from central Liberia across Ivory Coast, Ghana and Nigeria and into Cameroon and northern Gabon, in both in perhumid coastal forests and drier forests in the interior. It is phenotypically quite stable throughout with the notable exception of the Mount Swa population in Liberia where all green individuals were detected flying alongside typically patterned ones. Such an individual variation may be explained by phenotype selection to a heterogenous environment of patchy rain forest and savanna. Nevertheless, it remains to be confirmed that the green and typical morphs are indeed syntopic and we cannot exclude the possibility that the green morph represents a separate parapatric subspecies.

Euphaedra sarcoptera styx **Larsen & Warren-Gash, 2003**

http://species-id.net/wiki/Euphaedra_sarcoptera_styx
Figs 5E, 5F, 6E, 6F, 7E, 7F, 11C

Euphaedra sarcoptera styx Larsen & Warren-Gash, 2003: 97.

Type-locality. Tai Forest, Ivory Coast

Material examined. 1 ♂: Ivory Coast, Tai, 12.I.1999, H. Warren-Gash leg.; 3 ♂: Ivory Coast, Tai, 02.I.2000, H. Warren-Gash leg.; 1 ♂: Ivory Coast, Tai, XII.1998, K. Adams leg.; 1 ♀: Ivory Coast, Tai, 01–10.IV.2001, H. Warren-Gash leg., HWG; 1 ♂: Ivory Coast, Moyen Cavally, Tai Forest, IV.2001, prep. genit. 03/09.11.2012, J. Lorenc, HWG; 1 ♂: same data but 02.I.2000, MZUJ; 1 ♂: Tai, Ivory Coast, 13–14.VIII.2001, S. Collins; 1 ♂: same data but 13.IX.2001; 1 ♂: same data but 13–14.V.2000 (paratype *E. sarcoptera styx*); 1 ♂: Tai Forest, H. Warren-Gash leg., 12.1.1999 (paratype *E. sarcoptera styx*); 1 ♂: Tai Ivory Coast, 13–14.V.2000, S. Collins *leg*.; 1 ♀: same data but 13–14.VIII.2001, S. Collins leg., ABRI.

Diagnosis. Upperside ground colour black and pine green.

Redescription. Male (Figs 5E, 5F, 6E, 6F): Head, thorax and abdomen same to the nominate subspecies. Wings: FW (length 36–39 mm, mean: 37.8 mm, n=6) with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue, suffusing a black mid-discal cell black patch; pine green area from wing base to tornus, covering most of Cu2-1A and half of Cu1-Cu2; a large, elongated lawn green subapical patch, extending widely from subcosta costa to vein Cu1, with the basal limit marked by an incision along vein M3, and a diffused outer limit, not reaching outer margin; fringes grey and black. HWD oval with a gently scalloped outer margin; pine green grey except for the row of submarginal black, roughly rectangular patches, with a strong blue sheen gradually narrowing from roughly 4 mm at apex to cell Cu1-Cu2; fringes grey and black. FWV pine green, with a delicate golden overcast in subapical area; basal to mid-cell area pink; a series of black patches, a rounded postbasal spot, a wide mid cell bar, a similar bar along discal cell distal margin, four rectangular and one elongated patches in postdiscal area, a half-moon shaped patch in basal area of cell Cu1-Cu2, and a series of submarginal patches, five smaller from costa to cell M3-Cu1 and two larger, roughly oval, in Cu1-Cu2 and Cu2-1-2A. HWV golden yellow dusted with pine green in median area, with an elongated basal pink patch extending from costal margin to Rs to one-third costa, edged with black; six or seven median spots, four of which in discal cell, the one along costal margin considerably larger than the remainder, and a row of eight large, roughly oval black submarginal patches; marginal area darker pine green and black. *Male genitalia* (Fig. 11C): Tegumen same length as uncus, with a flat dorsal surface; uncus stout, aligned with the dorsal surface of shoulder, tip sharp, both tegumen and uncus longer than in the nominate subspecies; gnathos long, same length as uncus; pedunculus prominent; saccus flattened in lateral view; valvae with a smooth dorsal surface and blunt apex;

aedeagus as long as valvae tubular and straight, with a sharp distal extremity, slightly longer than in the nominate subspecies, and prominent cornuti.

Female (Figs 7E, 7F): Sexual dimorphism slight except for the larger size of the female (FW length: 40–41 mm, mean: 40.5 mm, n=2). *Female genitalia*: not examined.

Range. Known distribution is limited to the Tai National Park in western Ivory Coast.

Euphaedra sarcoptera ferrea Pyrcz & Warren-Gash, ssp. n.

http://species-id.net/wiki/Euphaedra_sarcoptera_ferrea
Figs 5C, 5D, 7C, 7D, 11E, 12B

Type-locality. Mount Nimba, Guinea

Material examined. Holotype ♂: Guinée (Guinea), Mont Nimba, VI.1998, ex HWG, MZUJ; Paratypes (7 ♂and 4 ♀): 5 ♂and 1 ♀: Guinée, Mont Nimba, VI.1998; 1 ♀: same data but VI.2000, HWG; 2 ♂: same data but 02.V.1998, ex HWG; 1 ♀: same data but 02.V.1998, ex HWG, prep. genit. 01/09.11.2012, J. Lorenc, 1 ♀: Ivory Coast, Azagny N. P., 13.VIII, 1999 (mislabeled), H. Warren-Gash leg., MZUJ.

Diagnosis. Upperside ground colour golden yellow, same as in *E. sarcoptera nipponicorum*, differing from the golden green of the nominate subspecies, pine green of *E. sarcoptera styx* or apple green of *E. sarcoptera cyparissoides*, differing from *E. sarcoptera nipponicorum*, widely separated geographically, only in the narrower FWD subcapical elongated golden yellow patch.

Description. Male (Figs 5C, 5D): Head, thorax and abdomen same as in the nominate subspecies. FW (length 38–40 mm, mean: 39 mm, n=6) with an elongated apical part, and gently convex outer margin; most of wing surface black, suffused with blue except for outer margin and apex, a black mid-discal cell black patch; golden green area from wing base to four-fifths of anal margin, covering most of discal cell; a large, elongated golden green subapical patch, extending from subcosta costa to vein Cu1, with the basal limit marked by an incision along vein M3, and a diffused outer edge, not reaching outer margin; fringes grey and black. HWD oval, compressed towards tornus, with a gently scalloped outer margin; golden green grey except for a black submarginal and marginal area, gradually narrowing from roughly 4 mm at apex towards tornus, where suffused with greenish blue scales; fringes grey and black. FWV pine green, lustrous, with a yellow overcast, except for the marginal area, more prominent in subapical area where forming a diffused elongated patch; basal to mid-cell area pink; a mid-cell bar, and a similar one along discal cell distal margin; four black, half-moon shaped patches along basally to the subapical yellow area, an elongated patch in mid cell Cu1-Cu2, and a series of submarginal patches, five smaller from costa to cell M3-Cu1 and two larger, oval, in Cu1-Cu2 and Cu2-1-2A. HWV golden yellow dusted with pine green along costa, with an elongated basal pink patch extending from costal margin to Rs to one-third costa, edged with black; six or seven median spots, four of which in discal cell, the one along costal margin considerably larger than the remainder; a row of eight large, roughly oval black submarginal patches; marginal area

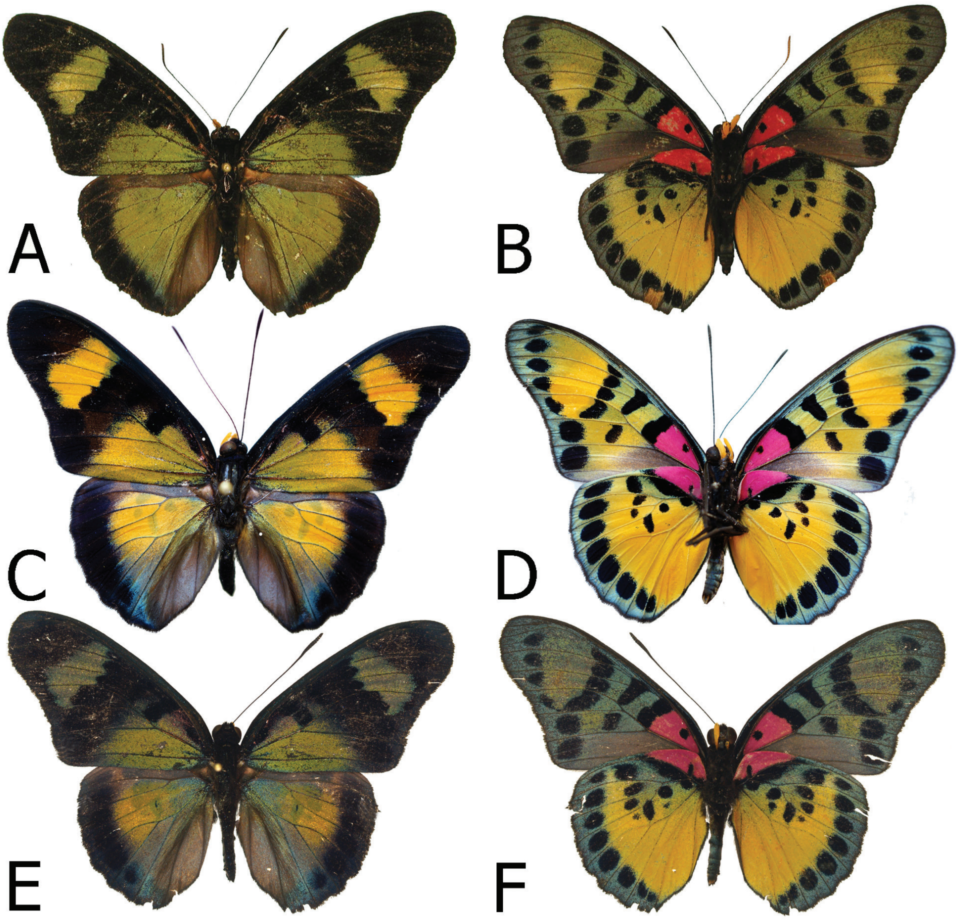

Figure 6. Adults, males: **A** *E. sarcoptera cyparissoides* holotype (dorsum) **B** *E. sarcoptera cyparissoides* holotype (venter) **C** *E. sarcoptera nipponicorum* Mbandaka, Congo (dorsum) **D** *E. sarcoptera nipponicorum* Mbandaka, Congo (venter) **E** *E. sarcoptera sarcoptera-styx* cline, Mount Swa, Liberia (dorsum) **F** *E. sarcoptera sarcoptera-styx* cline, Mount Swa, Liberia (venter).

pine green and black. *Male genitalia* (Fig. 11E): Tegumen same length as uncus, with a flat dorsal surface; uncus stout, slightly arched, with a sharp tip pointing downwards, tegument and uncus approximately the same length as in the nominate subspecies; gnathos long, same length as uncus; pedunculus prominent; saccus flattened in lateral view; valvae with a smooth dorsal surface with an elongated distal extremity ending with a sharp tip, differing in this respect from the blunt valvae of the nominate subspecies and *styx*; aedeagus as long as valvae tubular and straight, with a sharp distal extremity, sensibly longer than in the nominate subspecies or *styx*, and prominent cornuti.

Female (Figs 7C, 7D): Head, thorax and abdomen abdomen same as in the nominotypical subspecies. Wings: FW (length 42-46 mm, n=3) with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to

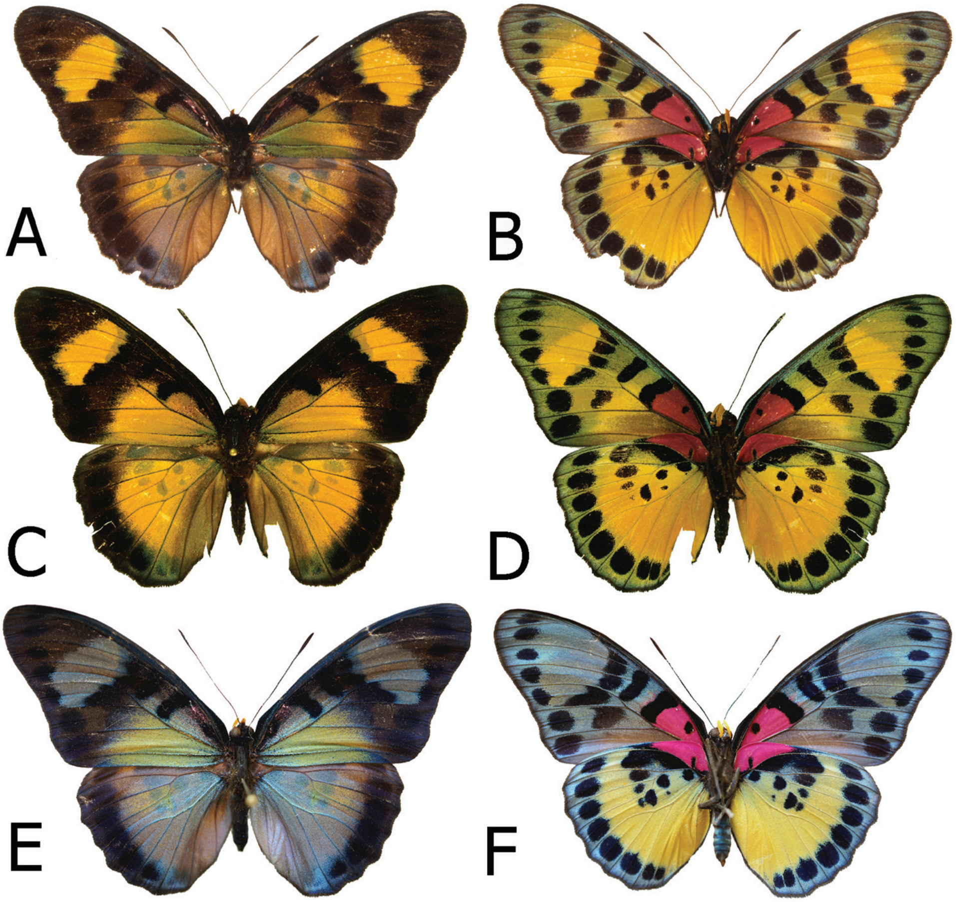

Figure 7. Adults, females: **A** *E. sarcoptera sarcoptera* Nsukka, Nigeria (dorsum) **B** *E. sarcoptera sarcoptera* Nsukka, Nigeria (venter) **C** *E. sarcoptera ferrea* Mont Nimba, Guinea, paratype (dorsum) **D** *E. sarcoptera ferrea* Mont Nimba, Guinea, paratype (venter) **E** *E. sarcoptera styx* Tai, Ivory Coast (dorsum) **F** *E. sarcoptera styx* Tai, Ivory Coast (venter).

apex dusted with dark blue; a golden yellow area from wing base to two-thirds of anal margin, covering lower half of discal cell; a prominent mid discal cell black round spot; a large, elongated golden yellow subapical patch, extending widely from subcosta costa to vein Cu1, with an irregular basal limit marked by an incision along vein M3, and a diffused outer limit, not reaching outer margin; fringes grey and black. HWD subtriangular with a gently scalloped outer margin; golden yellow, from Rs to costal margin dull brown; submarginal and marginal area black with a strong blue sheen, gradually narrowing from costa, where some 4 mm wide, to tornus, where blue takes over black; fringes grey and black. FWV green yellow, except for basal one-third where free of green and in subapical area where forming a large, diffuse patch; basal to median area purple; a series of black patches, a small rounded postbasal spot, a wide mid cell bar, a similar bar along discal cell distal margin, four patches along basal limit of a diffused subapical light yellow area, two outer triangular, two central rectangular, a large irregularly shaped patch in mid cell Cu1-Cu2, and a series of submarginal patches, five smaller from costa to cell M3-Cu1 and two larger, one rounded and one oval, in Cu1-Cu2 and Cu2-1-2A. HWV golden yellow, with an elongated basal purple patch extending from costal margin to Rs to one-fourth costa, edged with black; six black median spots, three of which in discal cell, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Female genitalia* (Fig. 12B): Bursa copulatrix very large, larger than the rest of genital body, oval, no signa; ductus bursae narrow, gradually widening towards the opening of bursa, one-third the length of bursa; colliculum narrow, slat like with folded edges, , one-third the length of ductus bursae; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis very small, slat-like; papillae anales three times as long as wide in lateral view, compressed in ventral view, apophyse posteriores one and half as long as the width of papillae anales; von Siebold organ prominent.

Etymology. The subspecific epithet of this taxon derives from iron in Latin, *ferrum*. The type locality, the massif of Mount Nimba on the Ivory Coast – Guinea border, is rich in iron ore. Iron ore mining is a serious threat for the unique ecosystem of this mountainous area of West Africa.

Range. Distribution limited to the Mount Nimba area. Considered that southern Ivory Coast was extensively sampled for several years, also by one of the authors (HWG), we believe that one paratype was labeled as from "Azagny" by error.

***Euphaedra sarcoptera cyparissoides* Hecq, 1979**

http://species-id.net/wiki/Euphaedra_sarcoptera_cyparissoides
Figs 6A, 6B

Euphaedra sarcoptera cyparissoïdes [sic] Hecq, 1979: 31.

Type-locality. Ketta Forest, Ouesso, Moyen Congo

Material examined. Holotype ♂: Ketta Forest (Ouesso), A.E.F. Moyen Congo, T. H. E. Jackon, VII.1959; 2 ♂: same data as the holotype, MRAC; 1 ♂: S Cameroon, Mintom, II.2009, P. A., ABRI.

Diagnosis. Upperside ground colour black and apple green.

Redescription. Male (Figs 6A, 6B): Head, thorax and abdomen same as in the nominate subspecies. Wings: FW (length 38–39 mm, n=2) with an elongated apical part, and gently convex outer margin; most of wing surface black almost completely outcasting a black mid-discal cell black patch; apple green area from wing base to three-fourth of anal margin, marginally entering the base of Cu2-1A and discal cell; a large, elongated apple green subapical patch a shade lighter, extending widely from subcosta costa to vein Cu1, with the basal limit marked by an incision along vein M3, and a diffused outer limit, not reaching outer margin; fringes grey and black. HWD oval, compressed towards tornus, with a gently scalloped outer margin; apple green

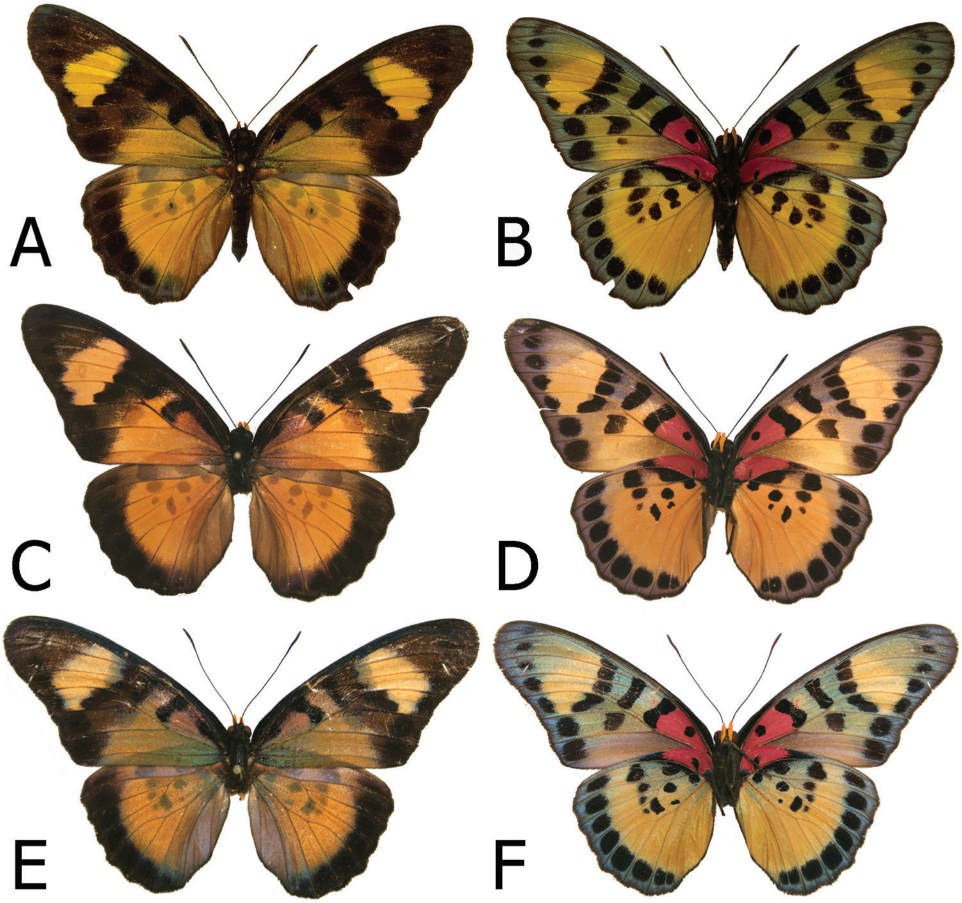

Figure 8. Adults, females : **A** *E. sarcoptera nipponicorum* Kigoma, Tanzania (dorsum) **B** *E. sarcoptera nipponicorum* Kigoma, Tanzania (venter) **C** *E. sarcoptera nipponicorum* Mbandaka, Congo (dorsum) **D** *E. sarcoptera nipponicorum* Mbandaka, Congo (venter) **E** *E. sarcoptera nipponicorum* Mbandaka, Congo (dorsum) **F** *E. sarcoptera nipponicorum* Mbandaka, Congo (venter).

grey except for the grey costal and black submarginal area gradually narrowing from roughly 4 mm at apex to cell Cu1-Cu2; fringes grey and black. FWV apple green, with a golden overcast in subapical area where forming a diffused patch; basal to mid-cell area pink; a series of black patches, a rounded postbasal spot, a wide mid cell bar, a similar bar along discal cell distal margin, four rectangular and one elongated patch in postdiscal area, a streak in basal area of cell Cu1-Cu2, and a series of submarginal patches, five smaller from costa to cell M3-Cu1 and two larger, roughly oval, in Cu1-Cu2 and Cu2-1-2A. HWV golden yellow dusted with apple green in median area, with an elongated basal pink patch extending from costal margin to Rs to one-third costa, edged with black; six or seven median spots, four of which in discal cell, the one along costal margin considerably larger than the remainder, and a row of eight large,

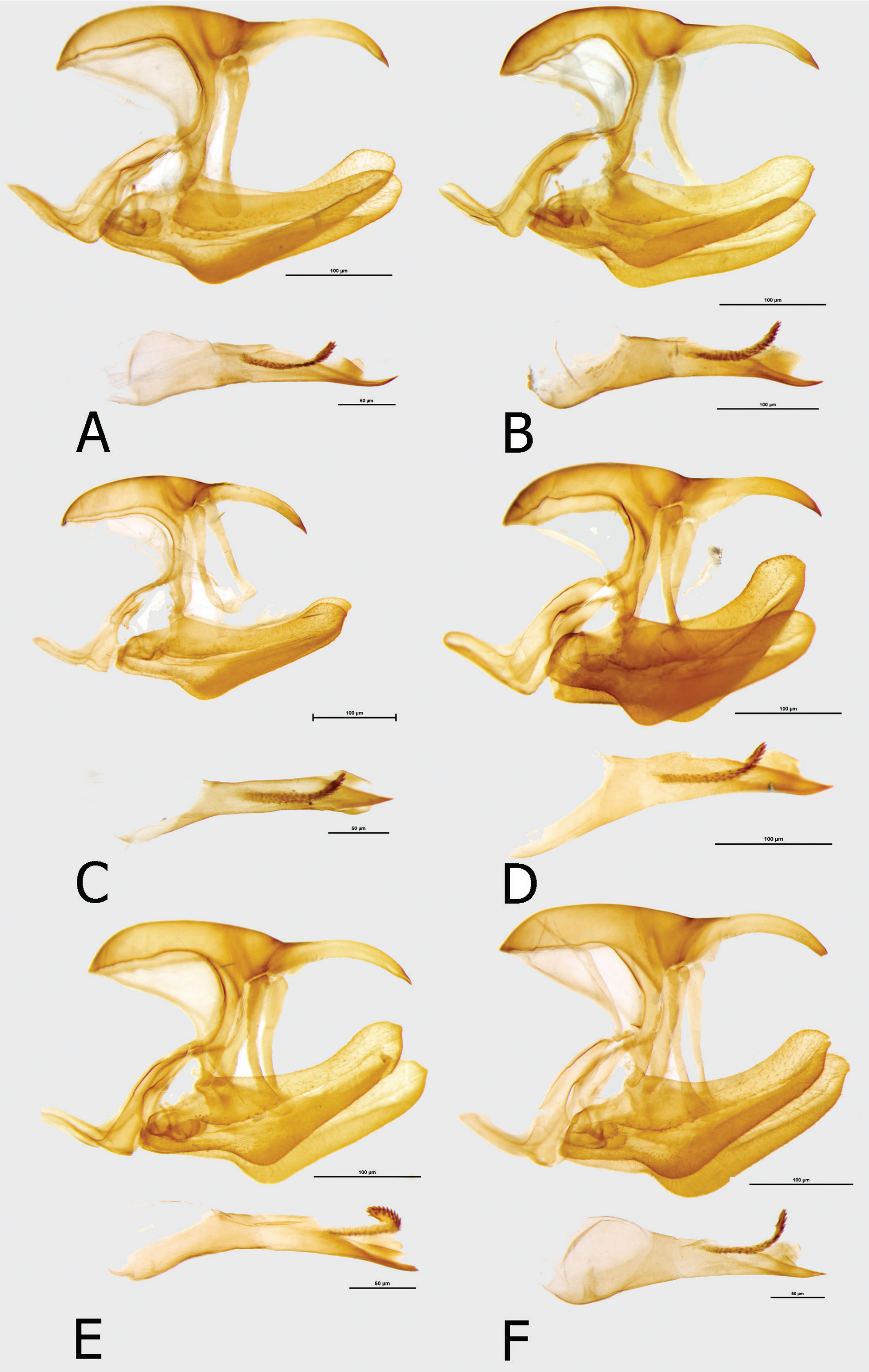

Figure 9. Male genitalia (in lateral view, aedeagus extracted): **A** *E. cyparissa cyparissa* Guma, Sierra Leone **B** *E. cyparissa tai* Tai, Ivory Coast **C** *E. cyparissa nimbina* Mont Nimba, Guinea **D** *E. cyparissa aurantina* Abengourou, Ivory Coast **E** *E. cyparissa aurata* Ebogo, Cameroon **F** *E. cyparissa nominalina* Bangui, Central African Republic.

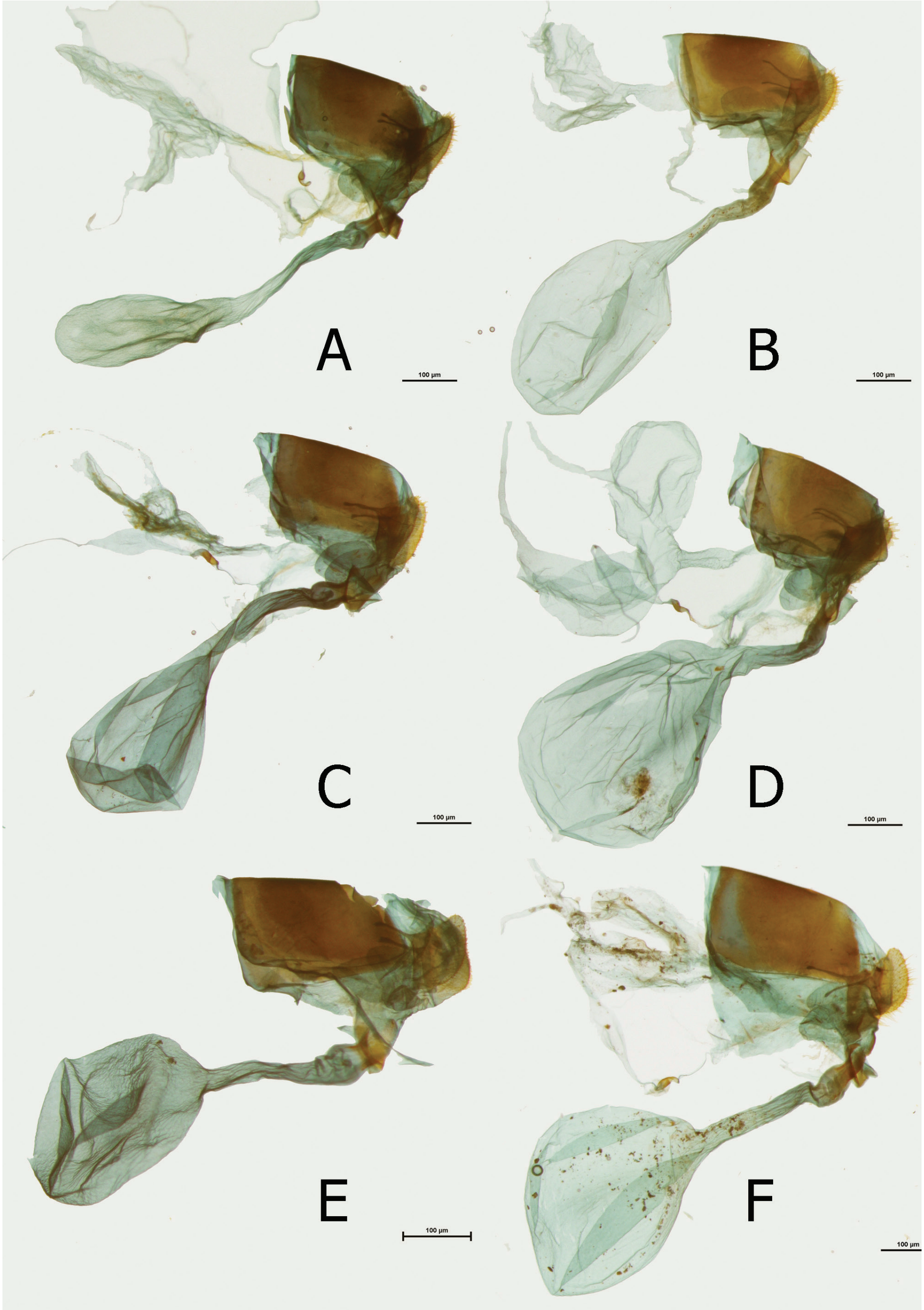

Figure 10. Female genitalia (in lateral view): **A** *E. cyparissa aurantina* Mpasso, Ghana **E** *cyparissa aurantina* Abengourou, Ivory Coast; 1 ♂: S. Nigeria, Ewohimi, 29.XI.55, J. Boorman; 1 ♂: no label; 2 ♂: Cameroon, Ebogo, Mbalmayo, X.2001; 1 ♂: Cameroon **C** *E. cyparissa nimbina* Mont Nimba, Guinea **D** *E. cyparissa tai* Tai, Ivory Coast **E** *E. cyparissa aurata* Obudu, Nigeria **F** *E. cyparissa nominalina* Mongorimba, R.C.A.

roughly oval black submarginal patches; marginal area dark green and black. *Male genitalia:* not examined.

Female: Not examined

Range. This subspecies was described from Ouesso in Congo, and apart from the holotype there are only a couple of other specimens known. Hecq (1979) mentions it from RCD and Cameroon. We could confirm its presence in SE Cameroon based on a specimen in ABRI. The reports from RDC might well refer to the next subspecies.

Euphaedra sarcoptera nipponicorum **(Carcasson), 1965**

http://species-id.net/wiki/Euphaedra_sarcoptera_nipponicorum
Figs 6C, 6D, 8A–F, 11B, 11D, 11F, 12A, 12C

Najas sarcoptera nipponicorum Carcasson, 1965: 132, fig. 4.
Euphaedra sarcoptera nipponicorum (Carcasson); Ackery et al. 1995: 414.

Type-locality. Itity e Camp, Mihuno, 25 miles east of Kigoma, Western Province, Tanzania.

Material examined. 3 ♀: Kasye Forest, Kigoma, Tanzania, III.1990, leg. local dealer, ex coll. P. Boyer, MZUJ; 3 ♂: R.C.A., Bangui, C.A.R., VIII.2000, S. Collins leg.; 1 ♂: R.C.A., Bangui, IX.2000, S. Collins leg., 2 ♂ and 1 ♀: R.C.A., Bangui, IV.2004, S. Collins leg.; 1 ♂: R.C.A., Bomoloto, R. Longamp, VII.1985, S. Collins leg.; 2 ♀: R.C.A., Bangui, VIII.2000, S. Collins leg.; 1 ♀: R.C.A., I.2004, S. Collins leg.; 2 ♂: R.D.C. (NW), Lukolela, Congo River, V.2012; 11 ♂ and 10 ♀: Tanzania, ABRI; 1 ♂: Congo, Province de l'Equateur, Mbandaka, 12.II.1998, prep. genit. 01/04.12.2012, J. Lorenc; 1 ♂: same data, prep. genit. 05/04.1.2012, J. Lorenc; 1 ♀: same data but XII.1997, prep. genit. 08/04.12.2012, J. Lorenc; 1 ♂: Congo, Province de l'Equateur, Environ de Mbandaka – Kuluboku, 27.IV.1995, prep. genit. 06/04.1.2012, J. Lorenc; 1 ♀: same data but 15.XI.1995, prep. genit. 07/04.12.2012, J. Lorenc; 1 ♂: Congo, Province de l'Equateur, Lukolela, 26.X.1993, prep. genit. 03/04.12.2012, J. Lorenc; 1 ♂: same data but 27.X.1993, prep. genit. 04/04.12.2012, J. Lorenc; 1 ♂: same data but XI.1993, PhO; 1 ♂: Sandoa, Katanga, R.D.C., Sandoa, 16.I .1991; 1 ♂: same data but 30.X.1920; 1 ♂: Kafakumba, Katanga, R.D.C., VII.1939; F.G. Overlaet leg.; 2 ♂: same data but II.1939; 1 ♂: same data but IX.1933; 1 ♂: Kinda, Katanga, R.D.C., 31.III.1916; 1 ♂: same data but 31.VIII.1916; 1 ♂: Montamba, 24.V.1916; 1 ♂: Kondué, East Kasai, R.D.C., no date, E. Luja leg.; 1 ♂: Tshilolo, Sankuru, East Kasai, 10.III.1951, Dr. Vallard leg.; 1 ♀: Kafakumba, Katanga, R.D.C., II.1939, F. G. Overlaet leg.; 1 ♀: same data but III.1939; 1 ♀: same data but IV.1939; 1 ♀: same data but IX.1933; 1 ♀: Kinda, 27.II.1916, F. G. Overlaet leg., MRAC.

Diagnosis. Upperside ground colour golden yellow, same as in *ferrea*.

Redescription. Male (Figs 6C, 6D): Head, thorax and abdomen same as in of the nominate subspecies. Wings: FW (length: 37–43 mm, mean: 40,3 mm, n=18) with an elongated apical part, and gently convex outer margin; most of wing surface black; costa from base to apex dusted with dark blue; a golden yellow area with a light green

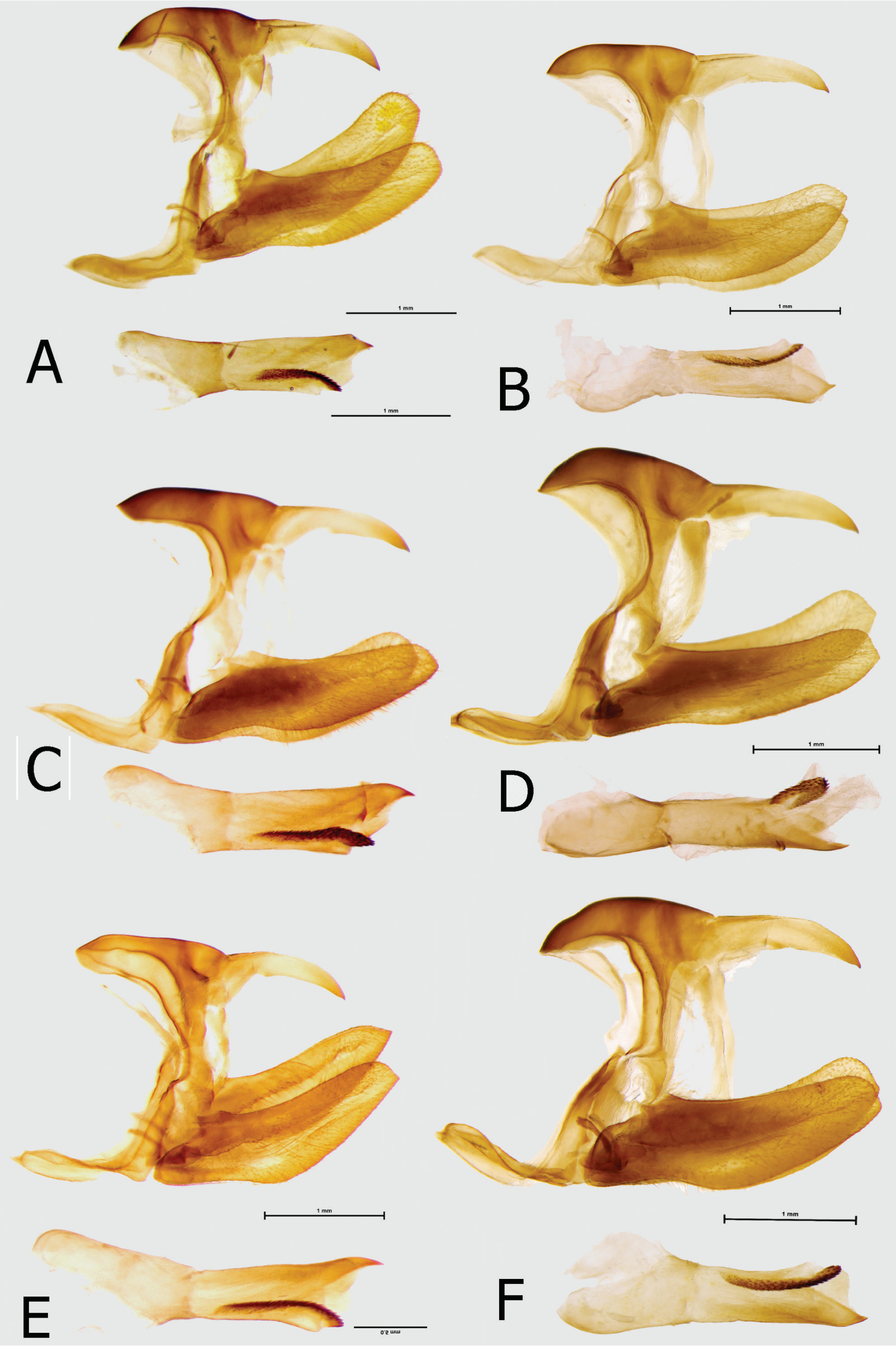

Figure 11. Male genitalia (in lateral view, aedeagus extracted): **A** *E. sarcoptera sarcoptera* Bibiani, Ghana **B** *E. sarcoptera nipponicorum* Mbandaka, Congo **C** *E. sarcoptera styx* Tai, Ivory Coast **D** *E. sarcoptera nipponicorum* Mbandaka, Congo **E** *E. sarcoptera ferrea* Mont Nimba, Guinea **F** *Euphaedra sarcoptera nipponicorum* Mbandaka, Congo.

suffusion from wing base to two-thirds of anal margin, covering lower half of discal cell; black postbasal and mid discal cell black round spots connected to black costal area; a large, elongated golden yellow subapical patch, extending from subcosta costa, progressively widening to space M2-M3, then narrowing and reaching to vein Cu1, with an irregular basal limit marked by an incision along vein M3, and a diffused outer limit, not reaching outer margin; fringes grey and black. HWD subtriangular with a gently scalloped outer margin; golden yellow, from Rs to costal margin dull brown; submarginal and marginal area black with a strong blue sheen, gradually narrowing from costa, where some 4 mm wide, to tornus, where greenish-blue takes over black and leaves one black spot in Cu1-Cu2 free; fringes grey and black. FWV green yellow, except for diffuse are in mid cell Cu2-1A-2A where free of green and in subapical area where forming a large patch shaped as on the upperside; basal to median area purple; a series of black patches, a rounded postbasal spot, a wide mid cell bar, a similar bar along discal cell distal margin, a series of four, moon-shaped patches along basal limit of a diffused subapical light yellow area, a large moon-shaped patch in mid cell Cu1-Cu2 coupled with a minute spot in Cu2-1A-2A, and a series of submarginal patches, five small from costa to cell M3-Cu1 and two large, roughly oval, in Cu1-Cu2 and Cu2-1A-2A. HWV golden yellow, with an elongated basal purple patch extending from costal margin to Rs to one-fourth costa, edged with black; six to seven black median spots, three or four of which in discal cell, and a row of eight large, roughly oval black submarginal patches; marginal area darker bottle green and black. *Male genitalia:* (Figs 11B, 11D, 11F) Tegumen same length as uncus, with a dome-like dorsal surface; uncus stout, more so than in other subspecies, slightly arched, with a sharp tip pointing downwards; gnathos long, same length as uncus; pedunculus prominent; saccus flattened in lateral view; valvae with a smooth dorsal surface with a blunt distal extremity ending with a small tip; aedeagus as long as valvae tubular and straight, with a sharp distal extremity, longer than in the nominate subspecies or *styx*, approaching the length of *nimbta,* and prominent cornuti.

Female (Figs 8A–F): Sexual dimorphism slight except for the larger size of the female (FW length: 40–51 mm, mean: 47,5 mm, n=10). *Female genitalia* (Figs 12A, 12C): Bursa copulatrix very large, larger than the rest of genital body, oval, no signa; ductus bursae wide, of same width throughout, one-third the length of bursa; colliculum short and weakly sclerotized; ductus seminalis joins the colliculum at the entrance of ductus bursae; lamella postvaginalis very small, slat-like; papillae anales three times as long as wide in lateral view, compressed in ventral view, apophyse posteriores one and half as long as the width of papillae anales; von Siebold organ prominent.

Range. This subspecies was described from the now largely destroyed Kasye forest in western Tanzania. It is however widely distributed across the Congo Basin south to Katanga (Shaba). The individuals coming from the area of Mbandaka in the western part of the Congo Basin are attributable to this subspecies. They present however an unusual, apparently individual variation in the ground colour of the upperside, ranging from orange-yellow, through typically golden yellow to yellow with a strong green overcast. Such an apparent individual variation, quite common in some species

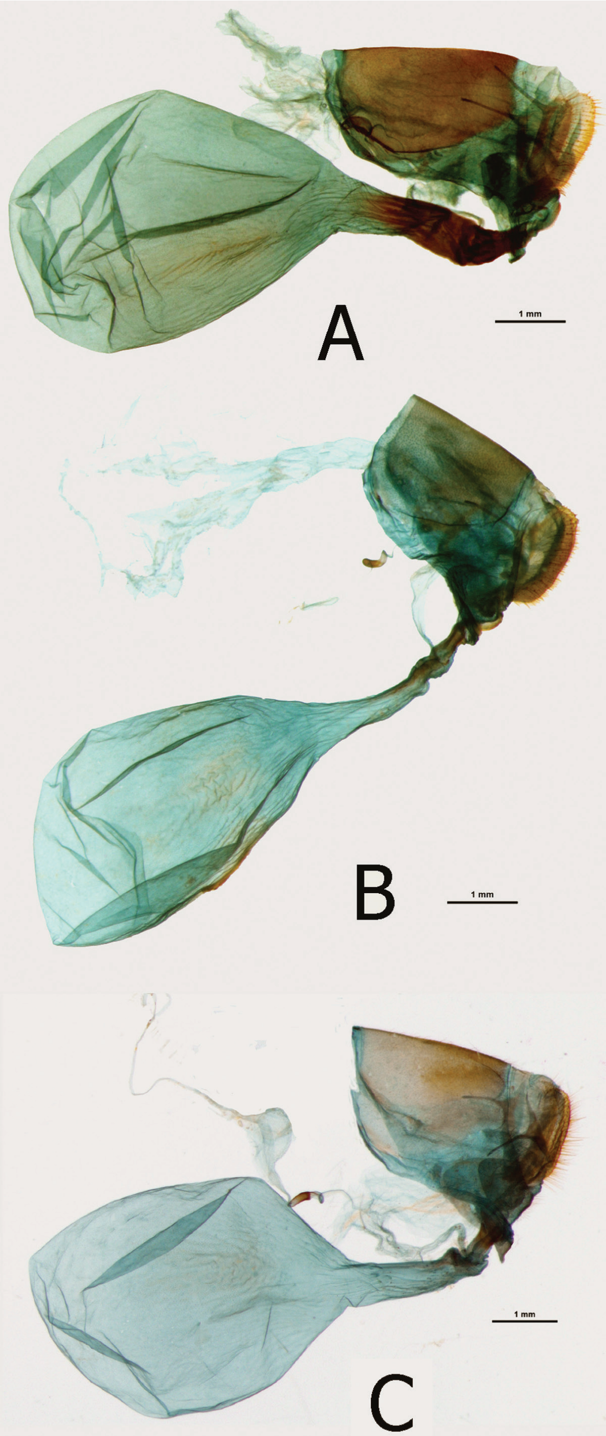

Figure 12. Female genitalia (in lateral view): **A** *E. sarcoptera nipponicorum* Kigoma, Tanzania **B** *Euphaedra sarcoptera ferrea* Mont Nimba, Guinea **C** *Euphaedra sarcoptera nipponicorum* Mbandaka, Congo.

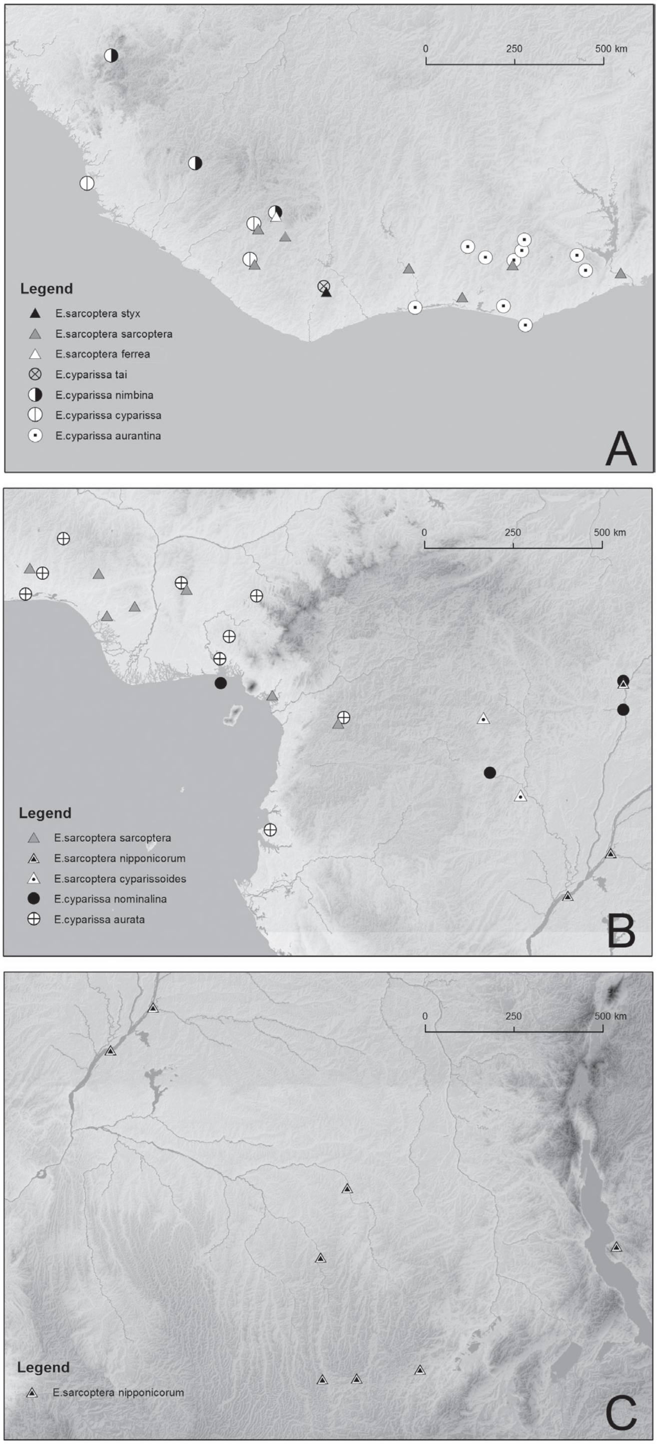

Figure 13. Distribution map: **A** West Africa, west of the Dahomey Gap **B** West Africa east of the Dahomey Gap, and central Africa **C** Congo Basin

of *Euphaedra sensu lato*, has been also observed in the Mount Swa population of *E. sarcoptera*. We refrain from further actions until more thorough sampling in the western Congo Basin confirms the differently patterned individuals are either syntopic or represent parapatric subspecies.

Discussion

Distribution and ecology

In terms of geographical range, the two species are largely sympatric, though, as noted above, *E. sarcoptera* stretches further south and east, into the Congo basin and the eastern shores of Lake Tanganyika. The six subspecies of *E. cyparissa* and the four of *E. sarcoptera* are discrete, in the sense of no overlap, and morphologically clearly distinct. Examination of the genitalia makes it clear that only two species are involved.

The apparent gaps in the range of *E. cyparissa* are puzzling. This is particularly striking in Nigeria where there are (or there were, since most of the western forests have now been logged down) strong populations in Yorubaland. There are however no records from the localities situated further south and east on the western bank of the river Niger. This is despite long-term and intensive collecting in the Okomu forest (Edo) by Wojtusiak, Knoop, Larsen and other researchers, or in Ajebandele (Ondo) by Pyrcz. East of the river Niger the pattern is equally unusual. Only one specimen was collected by Wojtusiak in Nsukka during five years of sampling. Larsen, however, collected several in the Oban Hills. Specimens are known from the Obudu range, and the riverine forests near Calabar. In Ghana and Ivory Coast the species seems to be widespread but local, distributed from Danané in north-western Ivory Coast to the Atewa Range in the Eastern Region of Ghana (Larsen 2006a; Larsen et al. 2007). Larsen (2005, 2006b) reports *E. cyparissa* from the Volta region but only as older records, probably from the collection of Father Theodor Maessen and we have not seen any specimens collected recently which would confirm its presence in the mountains on the border of Ghana and Togo. It is uncommon in eastern Sierra Leone and it has only recently been found in Liberia (Mount Swa), where it seems to be strongly localized. In its eastern range, *E. cyparissa* appears to be extremely scarce and local. In Cameroon, records are limited to two specimens from Ebogo, an intensively sampled locality, in the superb ABRI collection, while Vande Weghe (2010) reports it as very rare from a unique locality in northern Gabon. There are more frequent reports from CAR, however, compared to other *Euphaedra sensu lato* this species is far less frequently collected.

E. sarcoptera has a much wider geographic range but its distribution is also apparently disjunct. It is local and rare in Liberia (Mount Swa), while slightly commoner in some localities west of the Dahomey Gap, in particular in Ivory Coast and Ghana. However east of it, in southern Nigeria, with the exception of Sapoba, it is exceedingly rare, more so than its congener *E. cyparissa*. In fact, in four years of sampling by Wojtusiak in the forests of SE Nigeria only one specimen was collected, whereas the senior author (TP) despite of many years of collecting in the SW never came across it. Lees (1989) mentions it from Korup and most specimens in the BMNH are from the Niger Delta. Also in Cameroon *E. sarcoptera* is exceedingly seldom encountered. However, in some areas of the western Congo Basin and in Katanga it is locally rather frequently met. There are very few reports from the central Congo basin despite rather extensive collections of other species of *Euphaedra*.

In terms of habitat, the ecological preferences of *E. cyparissa* are inconsistent. Vande Weghe (2010) states briefly that *E. cyparissa* is a "deep forest" species, whereas Larsen (2005) considers it a rain forest edge, disturbed forest and even a gallery savannah species. He reports it "in small fragments of forests where few forest floor butterflies survive". It seems that both authors may in fact be correct. In our experience, most *E. cyparissa* have been collected on the fringes of secondary or primary mangrove and riverine forests in good condition, whether it be humid forest typical of the localities in Sierra Leone, or much of the Ivory Coast, or the drier inland forests of the eastern Ghana and Nigeria. The size of the forest is less important than the condition of what remains. Furthermore, and curiously, some populations of *E. cyparissa* appear to be lower montane, in particular *E. cyparissa nimbina* and the local population of *E. cyparissa aurata* found in the Obudu massif on the Nigeria-Cameroon border. *E. sarcoptera* occurs in similar habitats but Larsen (2005) points out that it is more strictly tied to forest than *E. cyparissa* which is possibly the case for West Africa. In the southern part of the Congo Basin however *E. sarcoptera* occurs apparently in riverine forests in a predominantly savanna land. Both species have recently been observed hill-topping on forested hilltops in Liberia (Mount Swa), a behaviour not reported for any other *Euphaedra*. Males perch rather high on leaves of trees in the lower canopy level or on the edge of forest gaps (6–15 m high) in the afternoon hours (14–15.00), they chase all passing *Euphaedra*, often involved in intraspecific and occasionally in interspecific interactions. The larval food plants of *E. cyparissa* and *E. sarcoptera* are unknown therefore their habitat preferences based on their hosts cannot be asserted.

Colour pattern convergence

It is interesting to observe that where the two species fly sympatrically, as they do through much of their range, the wing colour markings in one species are closely matched by the other. There are two plausible explanations for that. Either, it is a parallel evolution reflecting an adaptation to a particular forest light structure, a common background. In fact, all green populations are found in rain forest (*Euphaedra cyparissa tai, E. sarcoptera styx, E. cyparissa nominalina, E. sarcoptera cyparissoides*), the yellow ones (*Euphaedra sarcoptera nipponicorum, E. cyparissa ferrea, E. sarcoptera nimbina*) typically occur in drier and often more patchy forests. The second explanation would involve a mimicry scenario, implying that one of the species is the model, whereas the other the mimic. *Euphaedra* are not known to be obnoxious and unpalatable to predators, however the chemistry of this genus has not been investigated so far, so some level of toxicity cannot be ruled out. Such geographic pattern of colour convergence recalls classical examples of Müllerian mimicry among sympatric species and subspecies of many Ithomiines and *Heliconius* in the neotropical region. Pyrcz (in prep.) suggests another explanation for mimicry among palatable species, involving colour convergence to the most abundant pattern in a given area involving the process of anti-apostatic selection.

The validity of the subgenus *Euphaedra sensu* Hecq

Apart from the slender body and elongated wings, we did not find any other outstanding morphological characters in *E. cyparissa* and *E. sarcoptera* that would set them apart from other *Euphaedra sensu lato*. Male and female genitalia are typical of *Euphaedra sensu lato* compared to species of the "*ceres*" group for example (Pyrcz et al. 2011) or to the "*eleus*" group (Pyrcz et al., in prep.). The shape and the configuration of the vesica cornuti of *E. sarcoptera* and *E. cyparissa* do not present unequivocal, qualitative characters distinguishing the two species from other *Euphaedra sensu lato*. As between the two species, the external similarity of *E. cyparissa* and *E. sarcoptera* in terms of wing shape and colour patterns is striking. There are however significant differentiating characters, apart from the presence or absence of the pinkish basal patch on the FWV. In particular, the antennae of *E. cyparissa* are chestnut – orange on the underside while those of *E. sarcoptera* are black. In the male genitalia of *E. cyparissa,* the tegumen extends considerably basally, which is an exclusive character among the examined species of *Euphaedra sensu lato*, while in *E. sarcoptera* it is moderately elongated in the same way as in other species of *Euphaedra sensu lato*. The uncus of *E. cyparissa* is particularly slender and long while that of *E. sarcoptera* is average in length and thickness for species of *Euphaedra sensu lato*. In the female genitalia *E. cyparissa* and *E. sarcoptera* differ more from each other than the subgenus *Euphaedra sensu stricto* from *Euphaedra sensu lato*. The most noticeable character of *E. sarcoptera* is the particularly large corpus bursae and massive, yet short, ductus bursae, compared to the rather average in size homologous structures of *E. cyparissa*.

The most interesting trait of *E. cyparissa* and *E. sarcoptera* is arguably their unique behaviour, particularly striking for any naturalist with African rain forest experience. Whereas other *Euphaedra sensu lato* fly rapidly very close to the ground, *E. cyparissa* and *E. sarcoptera* flutter slowly, often several meters above the ground, and never keep to the understory. Larsen (2005) quotes Fermon's trapping result in Ghana where she collected many individuals in traps placed at 12 – 25 m above the ground in the forest canopy, which is justified by recent observations of hill-topping behaviour and displaying of males of both species on and above the lower canopy level. The senior author of this paper also observed the courtship behaviour of *E. cyparissa tai* in Tai National Park Forest (August, 1983), during which the two sexes flew up to some 3–5 metres above the rest of the group and engaged in a typical Nymphalid courtship behaviour (Thornhill and Alcock 1983).

Conclusion

In the light of this study, previously published articles and the ongoing research on the taxonomy of *Euphaedra sensu lato* (Pyrcz et al., in prep.), the morphological differences between the species in the subgenus *Euphaedra sensu* Hecq and other subgenera of *Euphaedra sensu lato* are insufficient alone to warrant it a separate taxonomic status. We found no characters in the external or genital morphology exclusive of *E. sarcoptera* and *E. cyparissa* which could be considered as solid qualitative synapomorphies sustaining its possible monophyly. However, there is a case for retaining the subgenus resting on behavioural grounds. The two species present unique features, involving territoriality and mating strategies, and the occurrence in the forest subcanopy. This study emphasizes as well the need for taking into account also other comparative and phylogenetically valuable characters than those so far available in the research on the genus *Euphaedra sensu lato*.

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References

Ackery PR, Smith LR, Vane-Wright RI (1995) Carcasson's African Butterflies. CSIRO, Australia, 803 pp.

Aurivillius C (1925) Die Afrikanischen Tagfalter (Ed. A. Seitz). Alfred Kernen, Stuttgart. 613 pp., 80 pl.

Amiet J-L (2004) Types de chenilles et de nymphes chez les *Euphaedra* du Cameroun (Lepidoptera, Nymphalidae, Limenitinae). Bulletin de la Société entomologique de France, 109(4): 333–359.

Butler AG (1866) Descriptions of six butterflies new to science, belonging to the genera, *Heterochroa* and *Ramaleosoma*. Proceedings of the Zoological Society of London, 1866: 43–59.

Butler AG (1873) Description of new species of Lepidoptera. Cistula Entomologica, 1 : 150–177.

Conceição IC, Long AD, Gruber JD, Beldade P (2011) Genomic Sequence around Butterfly Wing Development Genes: Annotation and Comparative Analysis. PLoS ONE 6(8): e23778. doi: 10.1371/journal.pone.0023778

D'Abrera B (2004) Butterflies of the Afrotropical Region. Part II - Nymphalidae, Libytheidae. Hills House Publishers, Melbourne-London, 356 pp.

Faravel G (2002) Description d'un *Euphaedra* nouveau du Gabon (Lepidoptera, Nymphalidae). Entomologia Africana, 7(2): 15–17.

Hecq J (1976) Eassai de classification du genre *Euphaedra* Hübner. Lambillionea, 76: 38–48, 53–63.

Hecq J (1979) Etude des *Euphaedra*. Clés de détermination des sous-genres *Euphaedra* Hübner et *Radia* Hecq. Lambillionea, 79: 31–32.

Hecq J (1984) Etude des *Euphaedra* (Nymphalidae africains). Monographie du groupe *preussi* (s.g. *Euphaedrana* Hecq). (Note no. 25). Lambillionea, 84: 9–16, 23–28.

Hecq J (1997) *Euphaedra*. Union des Entomologistes Belges, Tervuren, 117 pp.

Hecq J (1999) *Euphaedra* (and supplement in French). In: Bauer, E. and Frankenbach, T. Butterflies of the World, Part 4, Nymphalidae III. Goecke and Evers, Keltern, Germany.

Hecq J (2012) Catalogue des espèces d'*Euphaedra* sous-genre *Euphaedrana* Hecq 1976, groupe *eleus* et description de nouvelles espèces. Lambillionea, 112: 11–22.

Larsen TB (2005) Butterflies of West Africa, vol.1–2. Apollo Books, Stenstrup. vol.1, 595 pp, vol. 2, 270 pp.

Larsen TB (2006a) The Ghana butterfly fauna and its contribution to the objectives of the protected areas system. WDSP Report no. 63. Wildlife Division (Forestry Commission) and IUCN (World Conservation Union), Accra: 207 pp. http://abdb-africa.org/user/biblio/200620Ghana20Report20complete201.5MB.pdf

Larsen TB (2006b) The butterflies of Kyabobo National Park, Ghana, and those of the Volta Region. WDSP Report no. 64. Wildlife Division (Forestry Commission) and IUCN (World Conservation Union), Accra 60 pp. http://abdb africa.org/user/biblio/200620Kyabobo-Volta20complete20480K.pdf

Larsen TB, Aduse-Poku K, Boersma H, Sáfián Sz, Baker J (2007) Bobiri Butterfly Sanctuary in Ghana – Discovering its butterflies (with a checklist of the 930 butterflies of Ghana). Metamorphosis, 18(3): 87–126.

Lees DC (1989) A feasibility study into butterfly farming for the Korup National Park, Cameroun.

Monteiro AF, Pierce NE (2001) Phylogeny of *Bicyclus* (Lepidopterea: Nymphalidae) inferred from COI, COII, and EF-*a* gene sequences. Molecular Phylogenetics and Evolution, 18: 264: 281.

Pyrcz TW, Lorenc J, Knoop DP (2011) New species of *Euphaedra* Hübner of the ceres group from southwestern Nigeria – with new evidence from female genital morphology (Lepidoptera: Nymphalidae: Limenitidinae). Genus, 22(4): 621–638.

Razowski J (1996) Słownik morfologii owadów. PWN, Kraków, 434 pp.

Schultze A (1920) Lepidoptera II, in SCHUBOTZ, Al. Ergebnisse der zweiten Deutschen Zentral-Afrika Expedition, 1910–1911. Leipzig. Vol. 1, (Zoologie): 1113–1194.

Thornhill R, Alcock J (1983) The Evolution of Insect Mating Systems. Cambridge, MA: Harvard University Press.

Vande Weghe GR (2010) Papillons du Gabon. Wildlife Conservation Society, 424 pp.

Van Velzen R, Bakker FT, van Loon JJA (2007) DNA barcoding reveals hidden species diversity in Cymothoe (Nymphalidae). Proceedings of the Netherlands Entomological Society Meeting, 18: 95–103.

Van Velzen R, Larsen TB, Bakker FT (2009) A new hidden species of the Cymothoe caenis-complex (Lepidoptera: Nymphalidae) from western Africa. Zootaxa, 2197: 53–63.

Van Velzen R, Wahlberg N, Sosef MF, Bakker FT (2013) Effects of changing climate on species diversification in tropical forest butterflies of the genus *Cymothoe* (Lepidoptera: Nymphalidae). Biological Journal of the Linnaean Society. doi: 10.1111/bij.12012

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Revision of the genus *Placospongia* (Porifera, Demospongiae, Hadromerida, Placospongiidae) in the Indo-West Pacific

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Abstract

Species of the genus *Placospongia* are common within the tropical Indo-West Pacific, demonstrating a wide variety of colors and either branching or encrusting growth forms. A revision of Indo-West Pacific *Placospongia* was undertaken based on a redescription of the holotypes of species of *Placospongia* from the Indian Ocean and Western Pacific and an examination of an additional 103 specimens of *Placospongia* ssp. collected from Indonesia (including Vosmaer and Vernhout 1902 material), Seychelles, India, Singapore and Micronesia. One mitochondrial (COI) and one nuclear (ITS) marker were subsequently used to differentiate species. All *Placospongia* species are characterized by selenasters and tylostyles in two size classes. The combination of microsclere diversity and morphology as well as megasclere size were shown to be informative morphometric characters, supported by molecular evidence. Live coloration and growth form is shown to be unreliable for diagnoses. The study of holotypes found that *P. mixta* is a valid species and that two genus transfers are necessary: *Geodinella anthosigma* is a *Placospongia* and *P. labyrinthica* is a *Geodia*. A new species is also described from an anchialine pool in Indonesia, *Placospongia santodomingoae* **sp. n.**; bringing the total fauna of *Placospongia* species in the Indo-West Pacific to five: *Placospongia anthosigma, Placospongia carinata, Placospongia mixta, Placospongia melobesioides,* and *Placospongia santodomingoae* **sp. n.** An identification key is given. Two additional species, possibly morphologically cryptic, have been identified by molecular markers.

Keywords
Sponge, Indonesia, marine lake, coral reef, mangrove, anchialine pool, ITS, COI

Introduction

Species of the genus *Placospongia* in the tropical Indo-West Pacific occur in a wide variety of habitats such as marine lakes, coral reefs and mangroves. They may display a variety of colors and growth forms, from encrusting to branching (Figs 1, 2). Generally only two species have been recorded in species checklists within the Indo-West Pacific (e.g. Burton 1959, Hooper and Wiedenmeyer 1994, Hooper et al. 2000, Becking et al. 2006, de Voogd et al. 2008, de Voogd et al. 2009): *Placospongia melobesioides* Gray 1867, and *Placospongia carinata* (Bowerbank 1858). A recent collection of over 100 *Placospongia* specimens during fieldtrips by the author to Indonesia in 2006 (Sulawesi), 2007 (Papua), 2008, 2009 (Berau), and to Micronesia in 2010 (Yap) revealed, however, that there were more than two species present in these faunas.

The taxonomic literature records six valid species of *Placospongia* worldwide, of which there are three from the Indian Ocean and Western Pacific: *P. carinata* (type locality "South Sea", presumably in the Pacific), *Placospongia labyrinthica* Kirkpatrick

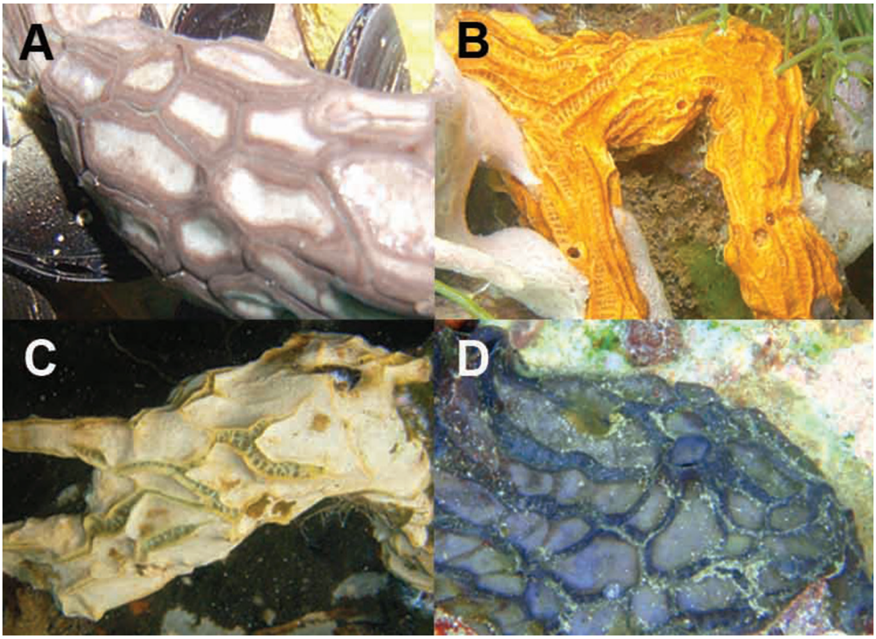

Figure 1. *In situ* underwater images of *Placospongia* spp. in Indonesia, displaying natural variation in color and growth form of live specimens. **A** *Placospongia mixta* (by L.E. Becking) **B** *Placospongia carinata* (by L.E. Becking) **C** *Placospongia carinata* (by L.E. Becking) **D** *Placospongia melobesioides* (by N.J. de Voogd).

1903 (type locality East London, South Africa, Indian Ocean), *P. melobesioides* (type locality Borneo, Pacific). In 1900 Thiele described the species *Placospongia mixta* from Ternate (Indonesia), which was later synonymized with *P. carinata* by Vosmaer and Vernhout in 1902. Vosmaer and Vernhout (1902) based their conclusions on a review of 26 specimens collected during the Siboga expedition to Indonesia, and this collection is presently housed at the Naturalis Biodiversity Center (Leiden, The Netherlands). Subsequently, according to the World Porifera Database (van Soest et al. 2011) *Geodinella anthosigma* Tanita and Hoshino 1989 (type locality Sagami Bay, Japan) should be transferred to the genus *Placospongia,* and *P. labyrinthica* should in fact be transferred to the genus *Geodia.* These suggested genus transfers have, however, not yet been published in the peer-reviewed literature. A molecular phylogeny constructed using the internal transcribed spacer region (ITS) indicated that there were nine evolutionary lineages worldwide within the genus *Placospongia* of which there were five distinct clades in the Indo-Pacific (clades C3, C4, C5, C6 & C9) that may represent five species (Nichols & Barnes 2005). The authors did not investigate the spicule morphology of the specimens in their study, therefore it is unclear which species name can be assigned to the different clades.

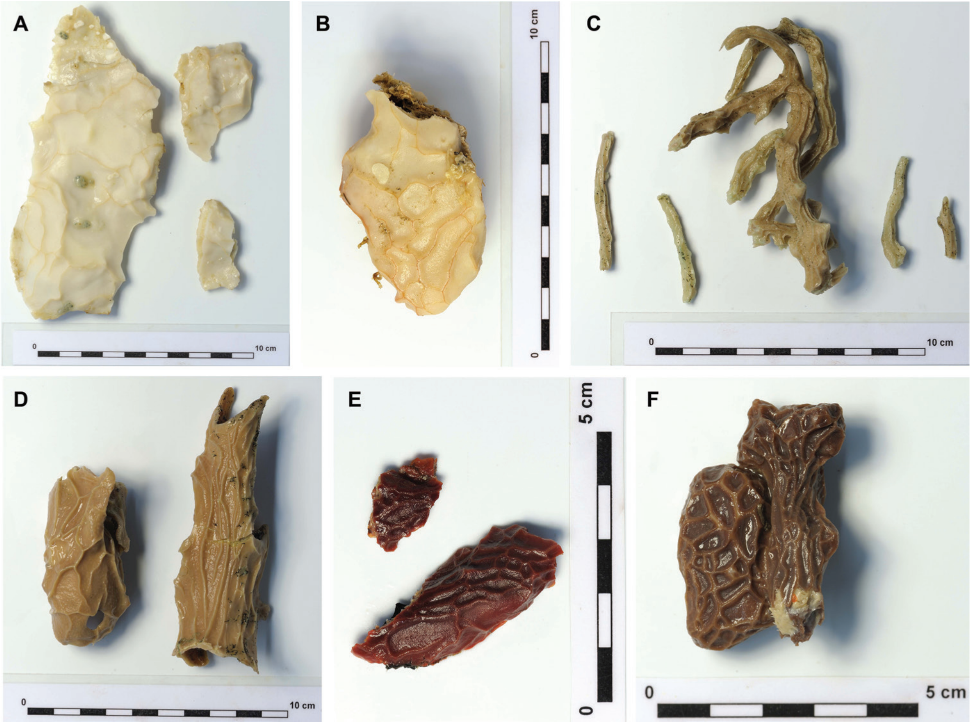

Figure 2. Gradation of external coloration in preserved specimens. **A** *Placospongia mixta* RMNH POR. 4492 **B** *Placospongia mixta* RMNH POR. 4113 **C** *Placospongia carinata* RMNH POR. 4482 **D** *Placospongia carinata* RMNH POR. 4483 **E** *Placospongia mixta* RMNH POR. 3979 **F** *Placospongia melobesioides* RMNH POR. 4114.

The objectives of the present study were to revise the genus *Placospongia* in the Indo-West Pacific by examining the holotypes of *P. melobesioides, P. carinata, P. mixta*, as well as 103 specimens of *Placospongia* spp. that were collected from Indonesia (including the Vosmaer & Vernhout material), Singapore, Seychelles, Madagascar, and Micronesia. In order to obtain a full view of the species from the Western Pacific and Indian Ocean the holotypes of the temperate species *G. anthosigma*, and *P. labyrinthica* were also examined. Subsequently it was determined if growth form and color can be used as diagnostic characteristics to identify different species of *Placospongia* in the field. Finally, the aim was to provide species names to the five clades of Indo-Pacific *Placospongia* as published by Nichols and Barnes (2005) by combining their published ITS sequences from GenBank with ITS sequences from identified species of Indo-Pacific *Placospongia.*

Material and methods

Specimens from Indonesia were collected via snorkeling in marine lakes and mangroves, and scuba diving in reefs. For a detailed description of marine lakes in Indonesia see Becking et al. (2011). Where possible material was preserved in 96% ethanol for DNA analysis, and voucher specimens were preserved in 70% ethanol and deposited in the collections of the Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH POR.). Records were made on the external morphology, skeletal architecture and spicules of all material. Spicule dimensions were measured of a subset of specimens indicated in Table 1, based on 25 measurements (unless noted otherwise) and given in the text as minimum-average-maximum. The following dimensions were measured: tylostyles length × shaft width × head width; selenasters length × width; streptasters total length × ray length; spherasters diameter; rhabds length × width. Only fully developed spicules were measured. To study the skeletal architecture hand-cut perpendicular sections of the choanosome were made. The sections were air-dried, mounted in Durcupan® ACM on a microscope slide, and studied under a Leica high power microscope. Spicule preparations were made by dissolving the organic tissue of a small fragment of the specimen in commercial bleach, after which the spicules were washed >10 times with distilled water and once with 96% ethanol. The spicules were air-dried on microscope slides and mounted with Durcupan® ACM. The spicules were also mounted on aluminium stubs, coated with gold-palladium and studied with a Jeol Scanning Electron Microscope.

DNA extractions were made with Qiagen DNEasy animal blood and tissue extraction kit following the manufacturer's protocol. The polymerase chain reaction (PCR) reaction volume was 25 *μ*l and contained 5 *μ*l Phire ® Hot Start reaction buffer, 1 unit Hotstart Phire® Hot Start DNA polymerase (Finnzymes), 2 *μ*l 1 mM dNTPs (Gibco), 1 *μ*l DNA template (5-20 ng) and 0.625 *μ*l of 10mM each primer. The standard DNA-barcoding fragment of the mitochondrial cytochrome oxidase subunit I (COI) fragment was amplified by using a specific forward primer designed by the author for

Table 1. Measurements of spicules of *Placospongia carinata, Placospongia melobesioides, Placospongia mixta,* and *Placospongia santodomingoae* sp. n. Sample location, growth form, color and spicule measurements provided per specimen. Spicule dimensions are based on 25 measurements and given in the text as minimum-average-maximum. Spheraster measurements in *P. melobesioides* based on less than ten measurements, due to low of abundance in specimens.

			tylostyle blunt end			tylostyle sharp end		
	growthform	**color live**	**length**	**max width**	**head width**	**length**	**max width**	**head width**
P. carinata								
R122b-86g-BK1390 (holotype)			500-**710.4**-800	10-**13.4**-15	10-**15.3**-18	140-**317.4**-450	5-**8.4**-12.5	8-**9.3**-13
RMNH POR. 4482	branching	orange	660-**726**-800	10-**12.3**-15	10-**14.5**-18	180-**263**-410	3-**5**-7.5	8-**7.5**-8
RMNH POR. 4483	encrusting	light brown	610-**703.8**-800	10-**13.1**-15	13-**14.9**-18	190-**286.7**-470	5-**6.4**-10	5-**8.6**-13
RMNH POR. 4484	encrusting	cream	560-**709.16**-920	8-**11.7**-18	10-**13.9**-18	175-**267.1**-550	3-**4.4**-10	5-**6.4**-13
RMNH POR. 4485	branching	dark brown	550-**761.2**-930	10-**14**-18	13-**15.5**-18	210-**295.2**-450	3-**5.6**-8	5-**7.6**-10
RMNH POR. 744	encrusting	purple	450-**748.6**-980	8-**11.1**-13	10-**13.2**-15	195-**256.8**-550	5-**6.2**-10	5-**6.7**-8
RMNH POR. 754	encrusting	white	540-**705.8**-830	10-**12.8**-15	13-**15.2**-18	280-**355.5**-500	5-**7.0**-10	5-**8.6**-13
RMNH POR. 755	encrusting	cream	560-**764.7**-910	8-**12.2**-15	10-**14.7**-18	250-**311.8**-360	5-**7.3**-8	5-**8.2**-10
ZMA Por. 10727	encrusting	-	620-**738.7**-840	8-**11**-13	13-**15.5**-18	240-**258.3**-270	3-**3.3**-5	3-**4.6**-8
ZMA Por. 9189	branching	-	550-**703.3**-820	10-**12.8**-15	13-**15**-18	210-**318.8**-410	5-**7.5**-10	5-**9.7**-13
P. melobesioides								
BMNH52.4.1.14 (holotype)	branching	dark brown	670-**879.6**-1010	10-**13.2**-18	10-**16.3**-20	205-**293.4**-420	5-**9.9**-13	5-**9.9**-13
RMNH POR. 4495	encrusting	dark brown	480-**717.6**-1040	5-**9.5**-15	8-**10.3**-15	190-**297.6**-370	3-**5.8**-8	3-**6.1**-8
RMNH POR. 4496	branching	dark brown	580-**778.4**-900	8-**11.7**-15	10-**14.1**-18	230-**272.8**-400	5-**7.4**-10	8-**9.1**-10
RMNH POR. 4497	branching	dark brown	620-**745.2**-860	10-**12.2**-15	13-**14.8**-18	250-**320.8**-450	5-**8.8**-10	5-**9.4**-13
RMNH POR. 3935	encrusting	dark brown	460-**660.9**-760	10-**11.6**-15	10-**13.7**-18	210-**325.8**-450	3-**7.4**-13	3-**8.3**-13
RMNH POR. 3166	encrusting	dark brown	460-**704.8**-810	8-**11.4**-13	10-**13.2**-15	200-**288**-470	3-**9.5**-13	5-**10.8**-15
RMNH POR. 3976	branching	dark brown	600-**793.6**-910	10-**12**-15	13-**14**-18	190-**321.2**-450	5-**8.5**-13	5-**9.6**-13
RMNH POR. 3977	branching	brown	510-**683.6**-780	10-**11.5**-13	13-**13.9**-15	200-**326**-450	5-**7.5**-10	8-**9.5**-13
RMNH POR. 758	branching	purple	630-**853.2**-1020	10-**13.3**-15	13-**15.8**-18	210-**253.2**-310	5-**9.5**-13	8-**11.8**-15
RMNH POR. 757	branching	white	550-**829.2**-960	10-**13.3**-16	13-**15.8**-18	260-**302.1**-370	8-**9.6**-13	10-**11.2**-15
RMNH POR. 2464	branching	-	710-**933.4**-1080	12.5-**15**-17.5	13-**15.7**-20	240-**326.7**-330	5-**9.2**-13	5-**10.8**-15
ZMA Por. 10459	branching	brown	520-**670.8**-820	7.5-**11.4**-12.5	10-**13.4**-17.5	310-**362.5**-430	5-**8.8**-10	5-**10.1**-13
P. mixta								
ZMB3204 (holotype)	encrusting	-	355-**672.4**-940	8-**12.1**-18	8-**15.6**-20	165-**226.4**-275	3-**6.1**-8	3-**7.8**-10

			tylostyle blunt end			tylostyle sharp end		
	growthform	color live	length	max width	head width	length	max width	head width
RMNH POR. 4112	encrusting	red	480-**870**-1040	10-**12.7**-15	13-**15.8**-28	210-**288**-410	5-**6.2**-10	5-**7.2**-10
RMNH POR. 4113	encrusting	cream	550-**817.6**-1030	10-**13.1**-15	13-**15.6**-18	160-**260**-350	5-**7.3**-10	5-**8.2**-12.5
RMNH POR. 742	branching	red	550-**759.2**-850	10-**11.9**-15	10-**14.9**-20	120-**230**-380	3-**5.9**-10	3-**7.6**-10
RMNH POR. 4489	encrusting	cream	630-**886.6**-1010	10-**12.9**-15	13-**15.4**-19	175-**221.5**-320	3-**3.9**-8	2-**7.2**-10
RMNH POR. 4490	encrusting	cream	510-**727.6**-970	8-13.120	13-**16.3**-23	150-**240**-310	3-**5.3**-8	2-**6.4**-8
RMNH POR. 4491	encrusting	brown	780-**1001.4**-1200	10-**14.8**-18	15-**17.5**-20	240-**284**-350	5-**6.3**-8	5-**8.3**-10
RMNH POR. 4492	encrusting	white	610-**995.8**-1250	10-**16**-20	13-**19**-25	260-**274**-290	8-**9**-10	8-**9**-10
RMNH POR. 3158	encrusting	cream	550-**990**-1210	13-**16.9**-20	13-**17.5**-20	130-**267.8**-400	5-**8.8**-15	8-**9**-10
RMNH POR. 745	encrusting	red	760-**914.1**-1030	13-**17**-23	10-**18**-25	250-**366.6**-480	3-**8**-13	3-**9**-13
RMNH POR. 4493	encrusting	brown	460-**761.6**-1070	10-**14.6**-23	13-**17.38**-25	220-**323.6**-430	8-**9.1**-13	10-**11.3**-15
RMNH POR. 4494	encrusting	brown	540-**758**-900	10-**12.2**-18	10-**13.8**-20	180-**216.9**-350	3-**3.3**-5	4-**4.4**-8
P. santodomingoae sp.n.								
RMNH POR. 4486 (holotype)	branching	brown	430-**605.6**-660	13-**15.5**-20	13-**18.1**-23	240-**261.3**-290	5-**7.2**-8	5-**8.8**-10
RMNH POR. 4487	branching	orange	530-**652.4**-740	13-**16**-20	15-**18.0**-23	220-**274.7**-310	5-**8.2**-13	8-**9.5**-15
RMNH POR. 4488	branching	orange	480-**633.2**-760	15-**17.2**-20	18-**19.6**-23	190-**273.2**-380	5-**7.9**-10	8-**10.3**-13

Table 1. Continued.

	selenaster		spheraster	streptaster		microrhabd	
	length	width	diameter	total length	length ray	length	width
P. carinata							
R122b-86g-BK1390 (holotype)	80-**90**-98	60-**71.3**-85		23-**33.8**-43	8-**11.6**-15	8-**12.0**-18	2.5
RMNH POR. 4482	65-**71.5**-75	50-**58.5**-65		15-**34**-48	10-**13.0**-15	8-**11.7**-15	2.5
RMNH POR. 4483	60-**80**-85	60-**62.9**-70		20-**33.7**-40	10-**13.2**-15	8-**11.9**-18	2.5
RMNH POR. 4484	50-**61.8**-70	35-**47.4**-55		25-**29.7**-35	8-**11.0**-15	10-**13.3**-18	2.5
RMNH POR. 4485	28-**63**-73	38-**50**-58		20-**27.6**-38	5-**9.0**-13	5-**9.4**-13	<2.5
RMNH POR. 744	60-**66.3**-70	50-**55.6**-65		25-**29.9**-38	10-12.918	8-**10.8**-13	<2.5
RMNH POR. 754	55-**67.7**-75	45-**51.8**-55		25-**30.9**-38	8-**9.5**-13	8-**12.3**-18	2.5
RMNH POR. 755	55-**61.1**-65	38-**47.5**-55		30-**32.9**-38	8-**9.8**-13	8-**10.2**-13	2.5
ZMA Por. 10727	50-**58.8**-78	35-**42.5**-63		25-**27.6**-38	8-**11.1**-15	8-**8.1**-10	<2.5

	selenaster		spheraster	streptaster		microrhabd	
	length	**width**	**diameter**	**total length**	**length ray**	**length**	**width**
ZMA Por. 9189	63-**72.2**-78	50-**56.8**-65		30-**35**-48	8-**10.7**-15	8-**9.2**-13	2.5
P. melobesioides							
BMNH52.4.1.14 (holotype)	58-**63.1**-68	45-**51.7**-68	15-**16.8**-18				
RMNH POR. 4495	45-**56.6**-70	30-**41.6**-50					
RMNH POR. 4496	45-**60**-75	35-**45**-63					
RMNH POR. 4497	63-**70.8**-83	45-**59.6**-65					
RMNH POR. 3935	45-**63.9**-70	38-**51.3**-60	15-20				
RMNH POR. 3166	60-**63.6**-70	50-**50.2**-55					
RMNH POR. 3976	48-**66.8**-75	48-**55.2**-65					
RMNH POR. 3977	58-**63.3**-68	40-**46**-53					
RMNH POR. 758	50-**55.2**-62.5	35-**42.3**-50	15				
RMNH POR. 757	55-**60.4**-65	43-**48.0**-53					
RMNH POR. 2464	67.5-**81**-87.5	60-**72.5**-85					
ZMA Por. 10459	62.5-**68.9**-72.5	50-**55.5**-65					
P. mixta							
ZMB3204 (holotype)	55-**69.8**-75	43-**55.4**-73	20-**25**-30	15-**23.9**-33	3-**7.6**-13	5-**7.1**-10	<2.5
RMNH POR. 4112	50-**66.6**-75	38-**50.7**-58	18-**20.2**-25	18-**23.7**-35	5-**6.4**-10	5-**6.4**-10	<2.5
RMNH POR. 4113	62.5-**66**-70	45-**53**-57.5	20-**22.1**-25	20-**24.8**-30	5-**5.7**-8	5-**7.5**-10	2,5
RMNH POR. 742	50-**65.4**-73	33-**46.5**-56	22-**23.4**-25	15-**22.2**-35	2-**5.7**-8	5-**7.4**-10	<2.5
RMNH POR. 4489	60-**68**-75	43-**50.8**-58	18-**20.6**-25	20-**26.1**-35	8-**10.8**-15	8-**8.5**-10	<2.5
RMNH POR. 4490	55-**70.4**-83	40-**53.3**-65	13-**20.5**-25	15-**21.7**-30	5-**6.4**-13	8-**9.2**-13	<2.5
RMNH POR. 4491	60-**71**-75	48-**57.5**-63	18-**23**-25	20-**27.3**-35	5-7-10	5-**6.3**-8	2,5
RMNH POR. 4492	58-**71**-78	45-**54.6**-70	15-**20.2**-25	18-**24.8**-33	10-**11.2**-15	5-**8.6**-18	<2.5
RMNH POR. 3158	65-**71**-75	50-**56.5**-63	23-**23.8**-25	23-**28.4**-35	5-**8.7**-13	5-**6.6**-8	<2.5
RMNH POR. 745	45-**73.6**-80	45-**60**-70	20-**23.9**-25	20-**23.7**-30	3-**6.4**-9	5-**7.5**-10	<2.5
RMNH POR. 4493	73-**80.3**-85	53-**65.3**-73	20-**26.5**-30	18-**23.4**-30	15-**8.1**-10	8-**8.7**-13	<2.5
RMNH POR. 4494	50-**59.1**-68	35-**42.3**-58	15-**20.9**-28	23-**26.9**-30	8-**10.4**-13	8-**8.5**-10	<2.5
P. santodomingoae sp.n.							
RMNH POR. 4486 (holotype)	80-**84.8**-90	60-**67.3**-75				8-**12.3**-18	2.5-**2.7**-3.5
RMNH POR. 4487	63-**82.9**-93	60-**66.3**-73				5-**10.5**-20	2.5-**2.6**-3.5
RMNH POR. 4488	80-**87**-93	58-**69**-75				8-**13.5**-18	2.5-**2.9**-3.5

Placospongia P-COI-F: GCA GG ATG ATA GGA ACA GGW TTT AG and the degenerated reverse primer from Folmer et al. (1994) designed by Meyer et al. (2005): dgHCO2198: TAA ACT TCA GGG TGA CCA AAR AAY CA. Temperature regime: 94°C for 30s; followed by 35 cycles of 94°C for 5s; 50°C for 5s; 72°C for 12 s; followed by 71°C for 1 min). ITS was amplified with primers from Wörheide (1998) RA2: GTC CCT GCC CTT TGT ACA CA and ITS2.2: CCT GGT TAG TTT CTT TTC CTC CGC). PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands). The poriferan origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were handled in SEQUENCHER 4.10.1 (Gene Codes Corporation) and aligned with CLUSTALW and MUSCLE implemented in DAMBE (Xia and Xie 2001). Species of the family Spirastrellidae were selected as outgroup for the phylogenetic analyses. For the COI genetree four specimens of *Spirastrella* aff. *decumbens* (RMNH POR. 4505, 4589, 4614) were taken. For the ITS genetree sequences of species from Spirastrellidae were taken from GenBank, as well as ITS sequences of Indo-Pacific *Placospongia* spp. from the study by Nichols & Barnes (2005), for GenBank accession numbers see Figure 11. The best-fit DNA substitution model was selected by the Akaike Information Criterion deployed in jMODELTEST v. 0.1.1 (Posada 2008) and this model (HKY for COI and GTR+G+I for ITS) was used for subsequent Bayesian and maximum likelihood phylogeny inferences. Phylogenetic reconstructions were performed under Bayesian inference criteria implemented in MrBayes v. 3.1.2. (Huelsenbeck and Ronquist 2001). Each analysis comprised two independent runs of four Metropolis-coupled Markov-chains, sampled at every 1000th generation at the default temperature (0.2). Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies <0.001. Convergence was also checked in Tracer v. 1.5.0 (Rambaut and Drummond 2007). For comparison, maximum likelihood bootstrap analyses were conducted using MEGA v. 5.01 (Tamura et al. 2011) using a heuristic search with 1000 bootstrap replicates. Within-group and between-group uncorrected *p*-distances were calculated in MEGA.

Abbreviations used in this manuscript: Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH POR.), the Zoological Museum of the University of Amsterdam (ZMA Por.), Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany (ZMB), The Natural History Museum, London, United Kingdom (BMNH).

Taxonomy

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Order Hadromerida Topsent, 1894
Family Placospongiidae Gray, 1867
Genus *Placospongia* Gray, 1867

Placospongia **Gray, 1867**

http://species-id.net/wiki/Placospongia

Type species: *Placospongia melobesioides* Gray, 1867 by monotypy

Description, amended from Systema Porifera (Hooper and van Soest 2002). Encrusting to branching growth forms. Small encrustations of 3 cm^2 to large surfaces of >2m^2 to branching individual with total size of up to 45cm in length and branch diameter between 0.25-1.5cm. Total size of specimens is hard to establish as parts of the body may be encrusting within cracks. Dried material is hard, alcohol preserved and live specimens remain compressible as the choanosome is of more pliant material than the cortex. The surface is made up of smooth cortical plates separated by contractible grooves which form a kind of network on the surface while these are firmly closed in preserved specimens. See Vosmaer & Vernhout (1902) and Rützler (2002) for an extensive description of the genus. In live specimens grooves are open and oscules are visible inside contractile ridges, running between plates. Live color white, cream, orange, reddish brown to dark black-brown (Fig. 1, 2) and come color is usually retained after alcohol preservation. The contact lines between the plates ridge up slightly and are generally a different shade of the color of the plates.

Skeleton. the cortical plates consist of densely packed selenasters and can also contain auxiliary microscleres. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates. In branching specimens radial tylostyle tracts run from the centre core (consisting of densely packed selenaster) to the cortical plates, in encrusting specimens tracts run in direction from substrate to cortex. The sharp ends of the smaller tylostyles are projected beyond the cortex surface. Microscleres occur in the cortex and scattered in choanosomal skeleton. For a detailed description of external morphology and anatomy see Vosmaer and Vernhout (1902).

Spicules. Megascleres are tylostyles in two size classes, microscleres are selenasters, and can include choanosomal and ectosomal spirasters (slender-spined streptasters and acanthose microrhabds), spherasters, and/or spherules. Selenasters often remain pigmented after treatment with bleach or nitric acid.

Placospongia anthosigma **(Tanita & Hoshino, 1989)**

http://species-id.net/wiki/Placospongia_anthosigma

Figure 3

Geodinella anthosigma Tanita & Hoshino, 1989: Fig. 16, Plate III Fig. 1

Material examined. Holotype. NSMT-Po R288 (National Museum of Nature and Science, Tokyo, Japan), Japan, Kannonzuka-dashi, Amadaiba, Sagami Bay, 62–67m. depth.

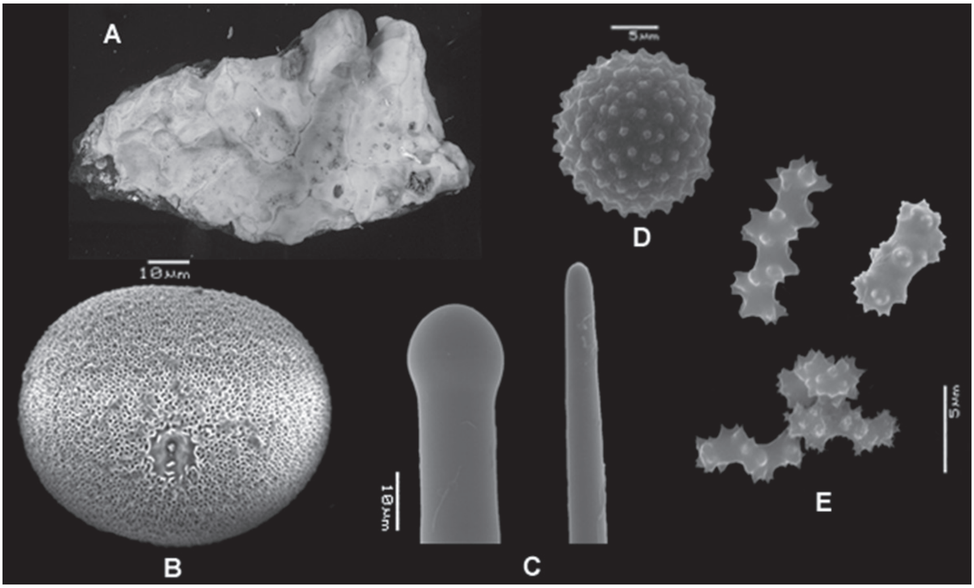

Figure 3. *Placospongia anthosigma* holotype (NSMT-Po R288) **A** type specimen (image taken from website database of the Museum of Nature and Science, Tokyo, Japan) **B** selenaster **C** large tylostyle (head and blunt end) **D** spheraster **E** spirasters referred to as 'anthosigma' by Tanita & Hoshino (1989).

Description. Holotype NSMT-Po R288 encrusting specimen in three pieces of 1–2cm^2 and 5mm thick, beige to pink in alcohol (Figure 3A).

Spicules. Megascleres large tylostyles with blunt point 520-797-930 × 15-18-20 × 18-20-23 μm, small tylostyles with blunt point 250-320-410 × 10-12-18 × 13-14-18 μm; microscleres selenasters 85-90-98 × 70-73-80 μm, spherasters 15-19-25 μm, stout spirasters with two or three contortions and acanthose spines spirally placed on shaft 8-11-18 × 3-4.5-5 μm (Fig. 3)

Skeleton. As description of genus with addition that spirasters form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spherasters amidst selenaster cortex and dispersed in choanosome.

Distribution. Type locality Sagami Bay, Eastern Japan, presently not recorded from any other locality.

Ecology. On rock substrate in deep temperate waters.

Remarks. Originally described by Tanita and Hoshino (1989) as *Geodinella anthosigma*. *Geodinella* is no longer a valid genus. *Geodinella anthosigma* should be transferred to the genus *Placospongia* based on the external morphology with the characteristic cortical plates and the presence of selenasters, tylostyles and spherasters. *Placospongia anthosigma* is distinguished from the other Indo-Pacific *Placospongia* spp. by the presence of contorted, spirally ornamented spirasters referred to by Tanita and Hoshino (1989) as 'anthosigma' and the small class of tylostyles with blunt points.

Placospongia carinata **(Bowerbank, 1858)**

http://species-id.net/wiki/Placospongia_carinata

Figures 4, 5

Geodia carinata Bowerbank, 1858: plate XXV Fig. 19.

Geodia carinata Bowerbank, 1874: plate XLVI Figs 1–5.

Material examined. Holotype. "South Sea": BMNH R1228 - 86g - Bk.1390 (slide), R1275 - PE01 - Bk1390 (slide).

Vosmaer and Vernhout (1902), Siboga expedition: RMNH POR. 755; RMNH POR. 754; RMNH POR. 744. **Other material:** RMNH POR. 4484, RMNH POR. 3943, RMNH POR. 3944, RMNH POR. 4485, RMNH POR. 3945, RMNH POR. 3946, RMNH POR. 3947, RMNH POR. 3948, RMNH POR. 3949, RMNH POR. 3950; RMNH POR. 3951, RMNH POR. 3952, RMNH POR. 3953, RMNH POR. 3954, RMNH POR. 3955, RMNH POR. 4482, RMNH POR. 3956, RMNH POR. 3957, RMNH POR. 4483, RMNH POR. 3958; ZMA Por. 8813ZMA Por. 09578; ZMA Por. 11367, ZMA Por. 16584, ZMA Por. 10727, ZMA Por. 1818, ZMA Por. 10481, ZMA Por. 20735; ZMA POR.9189. (See Table 2 for full details per specimen)

Description. Reviewed material is encrusting and/or branching. External morphology follows the description of the genus. Color of live specimens can be purple brown, chocolate brown, milk coffee brown, orange brown, orange, cream, or white (Fig. 1, 2). Color of choanosome is pale beige. After preservation in ethanol specimens retain some color of the live coloration.

Spicules. Holotype slide with spicules R1228-86g-Bk.1390 (BMNH) and slide with thick section R1275-PE01-Bk1390 (BMNH) (Fig. 4): megascleres large straight tylostyles with blunt ends 500-710-820 × 10-13-15 × 10-15-18 µm, small straight tylostyles with sharp ends 140-317-450 × 5-8-25 × 8-9-13 µm; microscleres selenasters 80-90-98 µm, streptasters with varying number of (spined) rays (5-10) with bifurcating endings or tufts 23-34-43 × 8-15 µm, acantho microrhabds 8-12-18 × 1-2.5 µm, spherasters absent. The range within the examined material (Table 1 & Fig. 5): megascleres large tylostyles 540-990 × 8-18 × 10-18 μm, small tylostyles 175-550 × 3-10 × 3-13 μm; microscleres selenasters 50-85 × 35-70 μm, streptasters 15-48 × 5-18 μm, acanthose microrhabds 5-18 × 1-2.5 μm, spherasters absent.

Skeleton. As description of genus with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spirasters scattered in choanosome.

Distribution. East African coast to eastern Indonesia (Fig. 9 , Table 2). Originally described from the 'South Sea', presumably the South Pacific Ocean. This has been interpreted by some (Rützler 2002, van Soest et al. 2011) to be Palau or Vanuatu, but this remains speculative. Based on the reviewed material and literature the minimal distribution is from Madagascar (Lévi 1956), to the Seychelles, and across Indonesia to the Aru Islands (Table 2). Distribution may extend further East.

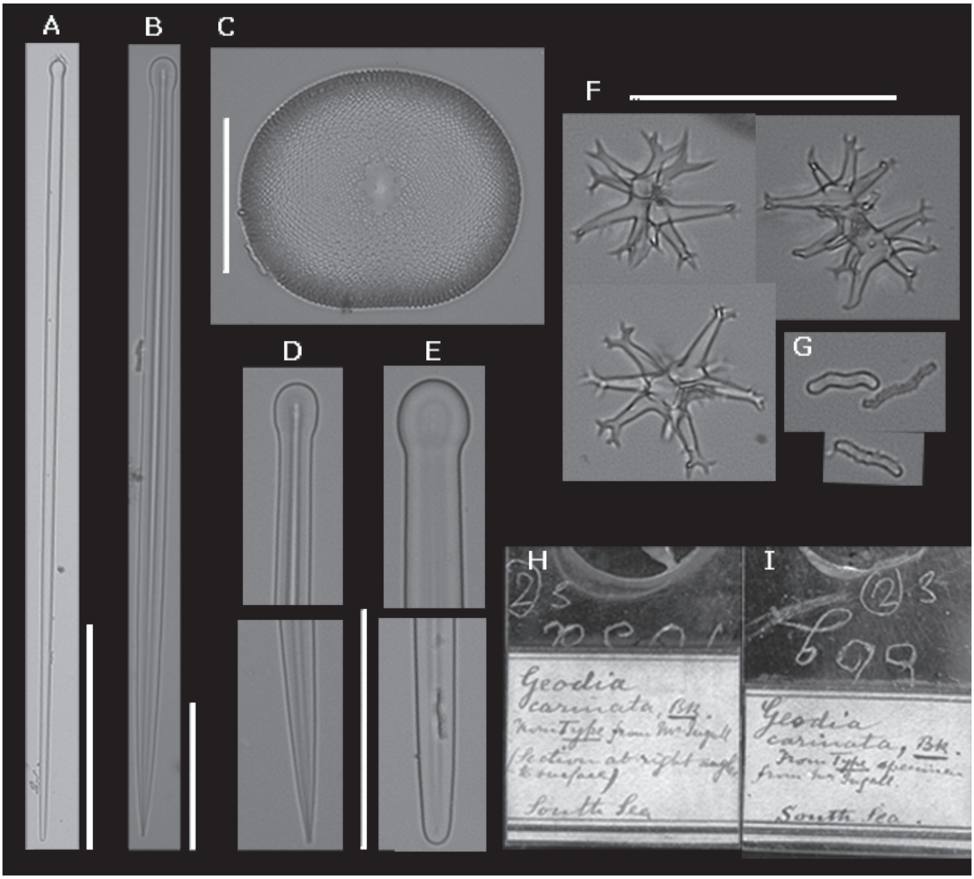

Figure 4. *Placospongia carinata* slide of holotype (BMNH, R1228, 86g, Bk.1390; R1275, PE01, Bk1390). **A** large tylostyle (scale=200 μm) **B** small tylostyle (scale=50 μm) **C** selenaster (scale=50 μm) **D** close up of large tylostyle (scale=50 μm) **E** close up of small tylostyle **F** streptasters (scale=50 μm) **G** acanthose microrhabds **H** original slide of thick section of holotype **I** original slide of spicules of holotype.

Ecology. Depth 0–45m. In Indonesia rarely found in reef environment, but high abundance in marine lakes. Possibly higher prevalence in reefs in Eastern Africa, based on the ZMA Por. collection from the Seychelles and the publication from Madagascar (Lévi 1956).

Remarks. The Bowerbank description from 1858 should be considered as the original description of '*Geodia carinata*', now accepted as *P. carinata,* with plates XXV Fig. 19 and XXVI Fig. 10 representing the streptasters ("arborescent elongo-subsphero-stella"). Subsequently in 1874 Bowerbank published a more extensive description of "*Geodia carinata"* including a drawing of the streptasters (Fig. 3, p.299) and spined microrhabds ("minute multiangulated cylindrical retentive spicula", fig.2, p.299) that he described as characteristic of the species. In neither

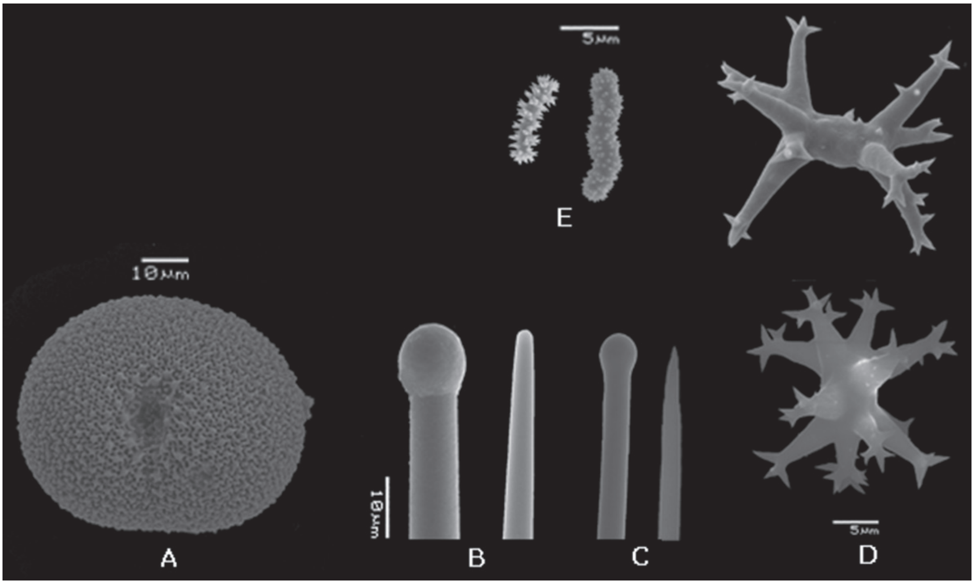

Figure 5. *Placospongia carinata* (RMNH POR. 4483). **A** selenaster **B** large tylostyle (head and blunt end) **C** small tylostyle (head and hastate end) **D** streptasters, E. acanthose microrhabds.

publication registration numbers were provided, however. The habitus drawing in Fig. 5, p299 of Bowerbank publication in 1874 is identical to the specimen BMNH95.6.7.1 that I received from the BMNH after requesting the holotype for *P. carinata*. In addition, I received the slides of spicules (codes: R1228, 86g, Bk.1390) and of the thick cut (codes: R1275, PE01, Bk1390) that were labeled to belong to the holotype (Fig. 5). Upon inspection I discovered that the specimen BMNH 95.6.7.1 is in fact a *P. melobesioides*, while the two slides do indeed represent *P. carinata* containing the characteristic streptasters with bifurcating endings and the microrhabds as indicated in the Bowerbank images and in the images taken from these slides in Fig. 5. The slides clearly do not come from the specimen BMNH 95.6.7.1. In the 16 years between Bowerbank's 1858 and 1874 publications, I fear that there has been some exchange or misinterpretation of the labels of the specimens resulting in the incorrect assignment of specimen BMNH 95.6.7.1 to the slides and as the holotype of *P. carinata*. This specimen BMNH 95.6.7.1, furthermore, has two labels attached to it: one with "*Geodia carinata*", and one with "*Placospongia melobesioides*". According to Bowerbank (1874) three specimens had been reviewed for his manuscript: one received from his friend Mr. Thos. Ingall in 1854, one placed by Dr. Baird from the coral to the sponge collection in the BMNH, and one specimen purchased by Bowerbank in 1864. The first mentioned specimen is presumably the holotype, but as this specimen has not been located, I propose to designate the slides R1228- 86g-Bk.1390 and R1275-PE01-Bk1390 as representing the holotype of *P. carinata*.

Table 2. Location details of reviewed specimens of *Placospongia carinata.*

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
RMNH POR. 744	#1500	Indonesia	Moluccas	W of Aru	Kur		benthic hard			20-40	6.xii.1899	Siboga expedition
RMNH POR. 754	#1458	Philippines		Sulu Sea	Ubian islands	anchorage off North Ubian	lithothamnion	06°7.5'N	120°26'E	23	28.vi.1899	Siboga expedition
RMNH POR. 755	#1848	Indonesia	West Papua	Raja Ampat	Misool		sand, stones	02°28'.5S	131°3'.3E	32	20.viii.1899	Siboga expedition
RMNH POR. 3943	#KKB/mol716	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3944	#KKB/mol754	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3945	#KKB/mol780	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3946	#KKB/mol810	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3947	#KKB/mol814	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3948	#KKB/mol825	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3949	#KKB/mol713	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3950	#KKB/mol1068	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3951	#MA/mol700	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3952	#MA/mol975	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3953	#MA/mol947	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
RMNH POR. 3954	#MA/mol1055	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3955	#MA/mol1012	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3956	#MA/mol1001	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3957	#MA/mol1009	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3958	#MA/mol1500	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4482	#MA/mol1061	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4483	#MA/LE172	Indonesia	East Kalimantan	Berau	Maratua	Haji Buang lake	marine lake	02°12'31.2"N	118°35'46.8"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4484	#KKB/mol110	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4485	#KKB/mol763	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
ZMA Por. 1818		Indonesia	Maluku		Banda islands	Banda anchorage	reef	04°32'23.3"S	129°54'28.8"E	9-45	22.xi.1899	Siboga expedition
ZMA Por. 9578		Singapore			Pulau Salu		reef	01°12'59.0"N	103°42'25.2"E	2	22.xii.1977	H. Moll
ZMA Por. 8813		Indonesia	Nusa Tenggara		Komodo	NE cape	reef	08°28'60.0"S	119°34'4.8"E	30	19.ix.1984	R.W.M. van Soest (Snellius II Expedition)
ZMA Por. 9189		India		Laccadive Islands	Agatti					20-25	1987	National Institute of Oceanography

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
ZMA Por. 10481		Seychelles		Mahé	Mahé	SE coast, near Pointe Cocos	reef			35-45	24.xii.1992	R.W.M. van Soest
ZMA Por. 10727		Seychelles		Mahé	Mahé	NE Point	reef	04°34'59.9"S	055°28'0.1"E	1	14.xii.1992	R.W.M. van Soest
ZMA Por. 11367		Seychelles		Mahé	N of Aride		reef	04°10'59.9"S	055°40'0.1"E	40	19.xii.1992	R.W.M. van Soest
ZMA Por. 16584		Seychelles		Mahé	Mahé	SW coast, Baie Lazare, Anse Gaulettes	reef	04°10'59.9"S	055°40'0.1"E	1-4	6.xii.1992	R.W.M. van Soest
ZMA Por. 20735		Seychelles		Mahé			reef				1992	R.W.M. van Soest

Placospongia melobesioides **Gray, 1867**

http://species-id.net/wiki/Placospongia_melobesioides
Figure 6

Placospongia melobesioides Gray (1867): Figs 1–4.

Material examined. Holotype. BMNH 52.4.1.14, Indonesia, Borneo island.

Vosmaer and Vernhout (1902), Siboga expedition: RMNH POR. 756, RMNH POR. 761, RMNH POR. 758, RMNH POR. 757, RMNH POR. 760, RMNH POR. 759. **Other material:**: RMNH POR. 4497, RMNH POR. 4496, RMNH POR. 4495, RMNH POR. 4114, RMNH POR. 3978, RMNH POR. 3977, RMNH POR. 3976, RMNH POR. 3942, RMNH POR. 3941, RMNH POR. 3940, RMNH POR. 3939, RMNH POR. 3938, RMNH POR. 3937, RMNH POR. 3935, RMNH POR. 3934, RMNH POR. 3933, RMNH POR. 3932, RMNH POR. 3177, RMNH POR. 3166, RMNH POR. 3154, RMNH POR. 2464, RMNH POR. 2463, ZMA Por. 13097, ZMA Por. 10459 (See Table 3 for full details per specimen)

Description. Holotype BMNH 52.4.1.14 dry, chalky white angular branches, hard. Other examined material encrusting to branching, hard, thicker specimens slightly compressible. External morphology follows the description of the genus. Size ranging between 5-50 cm, though encrusting specimens may be larger growing within crevices. Ectosome color in life ranging from purple, dark black brown, chocolate brown, orange brown to light beige (Fig. 1, 2). Choanosome pale beige. After preservation color of ectosome is similar to live color.

Spicules. Holotype BMNH 52.4.1.14 (Fig. 6): Megascleres large straight tylostyles with blunt ends 670-880-1010 × 10-13-18 × 10-16-20 µm, small concave to straight tylostyles with sharp ends 205-293-420 × 5-10-13 × 5-10-13 µm. Microscleres selenasters 58-63-68 × 45-52-68 µm, spherasters 15-17-18 µm (five measurements, not abundant), spherules 1-2-3 *µ*m. The range within the examined material (Table 1): large tylostyles 460-1040 × 5-16 × 8-18 *µ*m, small tylostyles 190-470 × 3-13 × 3-15 *µ*m, selenasters 45-83 × 30-65 *µ*m, spherules 1-3 *µ*m, spherasters only found in singles in some individuals 15-20 *µ*m. Streptasters and microrhabds absent.

Skeleton. As description of genus with addition of sporadic spherasters lodged amidst selenasters in cortex and high abundance of spherules in choanosome and cortex.

Ecology. Depth: 0-45m. Reefs, rocky shores, reefflats, mangroves, and marine lakes.

Distribution. Type locality: Borneo**.** Distribution from Seychelles to Micronesia (Fig. 9, Table 3). Possibly further east to Central Pacific.

Remarks. In the original description by Gray (1867) there is no mention of two size classes of tylostyles. I reexamined the original slide and conclude that the holotype does contain two size classes of tylostyles. The Systema Porifera indicates that the holotype has two size classes, the large 720-963-1200 × 13-14.1-19 µm and the small 350-438.8-560 × 8-9.1-10.5 µm, based on 10 measurements per spicule type (Rützler 2002). These measurements deviate from the holotype measurements in the present

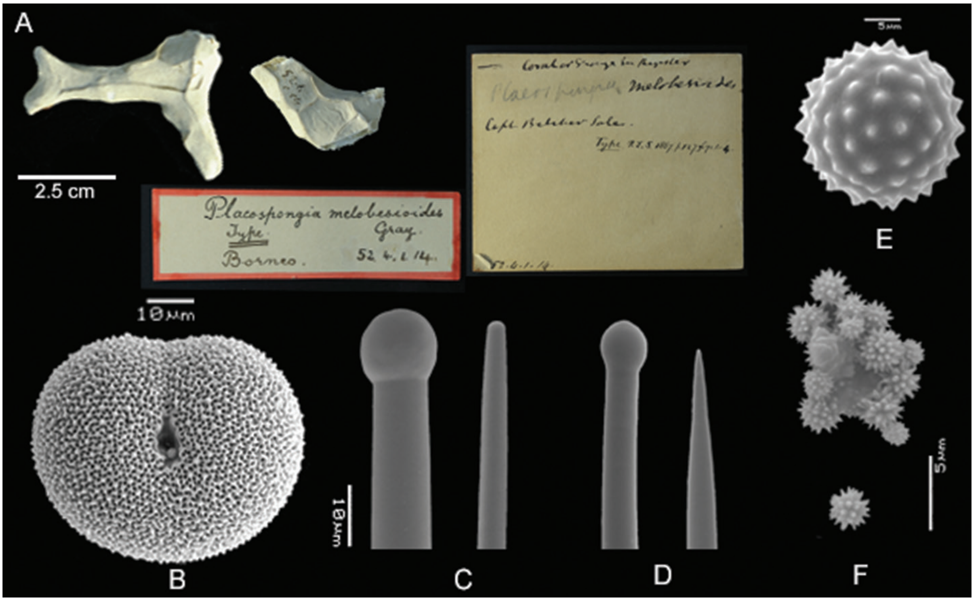

Figure 6. *Placospongia melobesioides* holotype (BMNH 52.4.1.14). **A** Holotype with two labels **B** selenaster **C** large tylostyle (head and blunt end) **D** small tylostyle (head and hastate end) **E** spheraster **F** spherules.

study that were based on 25 measurements per spicule type (670-880-1010 × 10-13-18 µm and 205-293-420 × 5-10-13 µm respectively), and also deviate from the range of sizes within the examined material of this study (Table 1). There is great variation in tylostyle length and spherasters are only sporadically present, often absent.

Placospongia mixta Thiele 1900

http://species-id.net/wiki/Placospongia_mixta

Figure 7

Placospongia mixta Thiele, 1900: Plate III, Fig. 25.

Material examined. Holotype. ZMB 3204, Indonesia, Moluccas, Ternate.

Vosmaerand& Vernhout (1902), Siboga expedition: RMNH POR. 753, RMNH POR. 751, RMNH POR. 745, RMNH POR. 742. **Other material:** RMNH POR. 4494, RMNH POR. 4493, RMNH, POR. 4492, RMNH POR. 4491, RMNH POR. 4490, RMNH POR. 4489, RMNH POR. 4113, RMNH POR. 4112, RMNH, POR. 3979, RMNH POR. 3975, RMNH POR. 3974, RMNH POR. 3973, RMNH POR. 3972, RMNH POR. 3971, RMNH POR. 3970, RMNH POR. 3969, RMNH POR. 3968, RMNH POR. 3967, RMNH POR. 3966, RMNH POR. 3965, RMNH

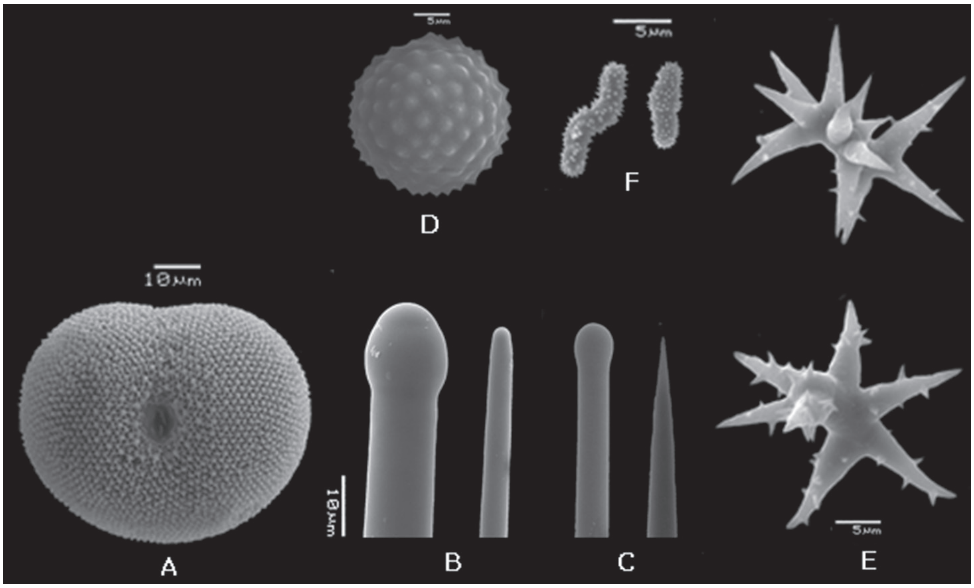

Figure 7. *Placospongia mixta* holotype (ZMB 3204). **A** selenaster **B** large tylostyle (head and blunt end) **C** small tylostyle (head and hastate end) **D** spheraster **E** streptasters **F** microacanthose microrhabds.

POR. 3964, RMNH POR. 3963, RMNH POR. 3962, RMNH POR. 3961, RMNH POR. 3960, RMNH POR. 3959, RMNH, POR. 3163, RMNH POR. 3158, RMNH POR. 3157, RMNH POR. 3155, RMNH POR. 3148, ZMA Por. 10495, ZMA Por. 896 (See Table 4 for full details per specimen)

Description. Holotype ZMB 3204 encrusting, size 5 × 2.5 cm and thickness 1–5 mm (as described by Thiele, now very small fragment), white after preservation in alcohol. The majority of the reviewed material is encrusting with a thickness of 4-10mm, but branching specimens also occur. External morphology follows the description of the genus. Color of the ectosome can be red, orange, brown orange, dark brown, chocolate brown, milk coffee brown, cream, or white (Fig. 1, 2). Color of choanosome is pale beige. After preservation in ethanol color is similar to live specimens, but lighter shade.

Spicules. Holotype ZMB 3204 (Fig. 6) Megascleres large straight tylostyles with blunt/rounded point 355-672-940 × 7.5-12-17.5 × 7.5-16-20 μm, small straight tylostyles with sharp point 165-226-275 × 2.5-6-7.5 × 2.5-8-10 μm; microscleres selenasters 55-70-75 × 42.5-55-72.5 μm, spherasters (abundant) 20-25-30 μm, streptasters typically with well developed axis and with 4-9 rays with hastate tips, rays are smooth or can be spined, but do not have bifurcations of the tips 15-24-32.5 × 2.5-8-12.5 μm; acanthose microrhabs with straight or zig-zag axis 5-7-10 × <2.5 *μ*m. The range within the examined material (Table 1): large tylostyles 460-1250 × 8-23 × 10-25 *μ*m, small tylostyles 120-430 × 3-15 × 2-15 *μ*m, selenasters 50-85 × 22-73 *μ*m, spherasters 13-30 *μ*m, streptasters 15-35 × 2-15 *μ*m, rays 5-18 × 1-2.5 *μ*m .

Table 3. Location details of reviewed specimens of *Placospongia melobesioides*.

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
RMNH POR. 761	#1033	Indonesia		S of Moluccas			sand & rock	04°12'S	129°20.4'E	45	1899	Siboga expedition
RMNH POR. 756	#660	Indonesia	Nusa Tenggara	N of Sumbawa			sand & rock	07°12.6'S	118°7.7'E	36	14.ii.1900	Siboga expedition
RMNH POR. 757	#1849	Indonesia	Moluccas	SE of Misool	Banda islands		sand & rock			32	1899	Siboga expedition
RMNH POR. 758	#1847	Indonesia	Moluccas	SE of Misool	Banda islands		sand & rock			32	1899	Siboga expedition
RMNH POR. 759	#1853	Indonesia	Moluccas	SE of Misool	Banda islands		sand & rock			32	1899	Siboga expedition
RMNH POR. 760	#1851	Indonesia	Moluccas	SE of Misool	Banda islands		sand & rock			32	1899	Siboga expedition
RMNH POR. 2463	#Sin05/270306/025	Singapore			Semaku	Pulau Semakau NW side	reef	01°13'70"N	103°45'61"E	10-12	iii.2006	N.J. de Voogd
RMNH POR. 2464	#Sin05/270306/026	Singapore			Semaku	Pulau Semakau NW side	reef	01°13'70"N	103°45'61"E	10-12	iii.2006	N.J. de Voogd
RMNH POR. 3154	#LEMD05/30	Indonesia	North Sulawesi		Bunaken	Pangalisang	reef	01°37'26"N	124°46'55"E	9	24.ix.2006	L.E.Becking
RMNH POR. 3166	#LEMD13/69	Indonesia	North Sulawesi		Bunaken	Pangalisang	reef	01°37'26"N	124°46'55"E	19	28.ix.2006	L.E.Becking
RMNH POR. 3177	#LEMD22/87	Indonesia	North Sulawesi		Bunaken	Likuan2	reef	01°35'78"N	124°46'06"E	21	13.x.2006	L.E.Becking
RMNH POR. 3932	#KKB/mol866	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3933	#KKB/mol766	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
RMNH POR. 3934	#KKB/mol767	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3935	#BER113/mol689	Indonesia	East Kalimantan	Berau	Maratua	NE Maratua	reef	02°17'32.3"N	118°35'26.1"E	5-10	15.viii.2008	N.J. de Voogd
RMNH POR. 3937	#BER107/mol604	Indonesia	East Kalimantan	Berau	Sangalaki	E Sangalaki	reef	02°05'36.6"N	118°24'15.2"E	5-10	15.viii.2008	L.E.Becking
RMNH POR. 3938	#BER107/mol608	Indonesia	East Kalimantan	Berau	Sangalaki	E Sangalaki	reef	02°05'36.6"N	118°24'15.2"E	5-10	15.viii.2008	L.E.Becking
RMNH POR. 3939	#BER108/mol601	Indonesia	East Kalimantan	Berau	Sangalaki	W Sangalaki	reef	02°05'07.7"N	118°23'28.0"E	5-10	15.viii.2008	L.E.Becking
RMNH POR. 3940	#P-YAP1	Micronesia	Yap		Yap		reefflat in front of mangrove	09°31'36.7"N	138°07'48.7"E	1-3	28.viii.2010	L.E.Becking
RMNH POR. 3941	#P-YAP2	Micronesia	Yap		Yap		reefflat in front of mangrove	09°31'36.7"N	138°07'48.7"E	1-3	28.viii.2010	L.E.Becking
RMNH POR. 3942	#P-YAP3	Micronesia	Yap		Yap		reefflat in front of mangrove	09°31'36.7"N	138°07'48.7"E	1-3	28.viii.2010	L.E.Becking
RMNH POR. 3976	#PM-TER02	Indonesia	Moluccas		Ternate		reef			5-10	xi.2009	N.J. de Voogd
RMNH POR. 3977	#PM-TER08	Indonesia	Moluccas		Ternate		reef			5-10	xi.2009	N.J. de Voogd
RMNH POR. 3978	#PM-TER12	Indonesia	Moluccas		Ternate		reef			5-10	xi.2009	N.J. de Voogd
RMNH POR. 4114	#KKB/mol795	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4495	#KKB/mol1075	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth (m.)	date	collector
RMNH POR. 4496	#KKB/mol776	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4497	#BER107/mol603	Indonesia	East Kalimantan	Berau	Sangalaki	E Sangalaki	reef	02°05'36.6"N	118°24'15.2"E	5-10	15.viii.2008	L.E.Becking
ZMA Por. 10459		Seychelles	Mahé		Mahé	NE coast, North East Point	reef	04°34'59.9"S	055°28'0.1"E	5	8.xii.1992	R.W.M. van Soest
ZMA Por. 10496		Seychelles	Mahé		Mahé	North East Point	reef	04°34'59.9"S	055°28'0.1"E		14.xii.1992	R.W.M. van Soest
ZMA Por. 13097		Indonesia	South Sulawesi	Spermonde archipelago	Samalona		reef			5-30	27.iv.1997	N.J. de Voogd

Skeleton. As description of genus with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Streptasters scattered in choanosome. Spherasters amidst selenasters in cortex and scattered in choanosome.

Distribution. East African coast to eastern Indonesia (Fig. 9, Table 4). Possibly further east to Central Pacific. Pulitzer-Finali (1993) identified a '*P. carinata'* from East Africa (Mombasa) that fits the description of *P. mixta* based on the length of the tylostyles (up to 1200 *μ*m) and the presence of spherasters, but no *P. mixta* specimens were observed in the Seychelles material deposited at ZMA.

Ecology. Depth 0–45m. Common in reefs, also occurs in marine lakes.

Remarks. In 1900 Thiele described a new species named *P. mixta*, which was originally identified as *P. melobesioides* by Kieschnick (1896). The specific epithet *mixta* was given because the specimen contained a mixture of spicules: both spirasters like *P. carinata* as well as large spherasters like *P. intermedia* and *P. melobesioides*, which are absent in *P. carinata*. In 1902 Vosmaer & Vernhout decided that *P. mixta* was a junior synonym of *P. carinata*, because they saw no distinction between the different shapes of streptasters and stated that spherasters are never very abundant – in some 'exceedingly rare and in some we failed to find them at all' – and could therefore not be seen as a distinguishing character. The specimens that were studied by Vosmaer and Vernhout (1902) were collected in Indonesia during the Siboga Expedition (1899-1900) and are housed in the collection of the Naturalis Biodiversity Center (Leiden, The Netherlands). In the present study these specimens were reexamined. After inspection, the specimens labeled '*P. carinata*' could be clearly and consistently divided into two species: *P. carinata* without spherasters, with streptasters displaying bifurcating tips, and tylostyles up to 980 *μ*m, and *P. mixta* with abundant spherasters, with streptasters displaying hastate tips, and tylostyles up to 1250 *μ*m. In none of the specimens of Vosmaer & Vernhout (1902), nor of the other specimens reviewed for this study was there a mixture of the two types of streptasters. These two species also show molecular distinction in both mitochondrial and nuclear markers (Fig. 10, 11, Table 6, 7).

Placospongia santodomingoae sp. n.

urn:lsid:zoobank.org:act:3C4F2599-15C0-4075-BD3B-8C6439C8F821
http://species-id.net/wiki/Placospongia_santodomingoae
Figure 8

Holotype. RMNH POR. 4486, Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, 02°11'16.4"N, 118°37'06.4"E, 0–1m. depth, xi.2008, coll. N.K. Santodomingo & Estradivari, #BER128/mol1147. **Paratypes.** RMNH POR. 4487, Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, 02°11'16.4"N, 118°37'06.4"E, 0–1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari; RMNH POR. 4488, Indonesia, East Kalimantan province, Marat-

Table 4. Location details of reviewed specimens of *Placospongia mixta*.

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth	date	collector
RMNH POR. 753	#311	Indonesia	West Papua	E. of Misool			sand & rock	01°42.5'S	130°47.5'E	32	20.viii.1899	Siboga expedition
RMNH POR. 751	#1857	Indonesia	West Papua	E. of Misool			sand & rock	01°42.5'S	130°47.5'E	32	20.viii.1899	Siboga expedition
RMNH POR. 745	#577	Indonesia	South Sulawesi		N. of Kabia	Saleyer anchorage	sand & rock			36	20.viii.1899	Siboga expedition
RMNH POR. 742	#163a	Indonesia	Moluccas		Aru	Pearl Banks, anchorage off Pulu Jedan	reef			13	23.xii.1899	Siboga expedition
RMNH POR. 3148	#LEMD04/21	Indonesia	North Sulawesi		Bunaken	Likuan 2	reef	01°35'78"N	124°46'06"E	15	24.ix.2006	L.E. Becking
RMNH POR. 3155	#LEMD06/32	Indonesia	North Sulawesi	Lembeh Strait		Nudi Reed Reed	reef	01°24'06"N	125°12'22"E	21	25.ix.2006	L.E. Becking
RMNH POR. 3157	#LEMD08/39	Indonesia	North Sulawesi	Lembeh Strait		Nudi Fols	reef	01°27'26"N	125°13'05"E	6	25.ix.2006	L.E. Becking
RMNH POR. 3158	#LEMD08/42	Indonesia	North Sulawesi	Lembeh Strait		Nudi Fols	reef	01°27'26"N	125°13'05"E	8	25.ix.2006	L.E. Becking
RMNH POR. 3163	#LEMD11/52	Indonesia	North Sulawesi		Bunaken	0.5-1km W. of Park administration office	reef	01°36'57"N	124°45'41"E	8	27.ix.2006	L.E. Becking
RMNH POR. 3959	#KKB/mol827	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3960	#KKB/mol829	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3961	#KKB/mol851	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 3962	#BER111/mol1203	Indonesia	East Kalimantan	Berau	Kakaban	SW Kakaban	reef	02°08'07.5"N	118°30'23.3"E	10	17.viii.2008	N.J. de Voogd

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth	date	collector
RMNH POR. 3963	#BER111/1209	Indonesia	East Kalimantan	Berau	Kakaban	SW Kakaban	reef	02°08'07.5"N	118°30'23.3"E	10	17.viii.2008	N.J. de Voogd
RMNH POR. 3964	#BER111/1213	Indonesia	East Kalimantan	Berau	Kakaban	SW Kakaban	reef	02°08'07.5"N	118°30'23.3"E	10	17.viii.2008	N.J. de Voogd
RMNH POR. 3965	#BER111/ mol1219	Indonesia	East Kalimantan	Berau	Kakaban	SW Kakaban	reef	02°08'07.5"N	118°30'23.3"E	10	17.viii.2008	N.J. de Voogd
RMNH POR. 3966	#RAJ23/ mol195	Indonesia	West Papua	Raja Ampat	Gam	Ctenophore lake	marine lake	00°27'17.5"S	130°29'33.8"E	0-2	xi.2007	L.E. Becking
RMNH POR. 3967	#RAJ23/ mol187	Indonesia	West Papua	Raja Ampat	Gam	Ctenophore lake	marine lake	00°27'17.5"S	130°29'33.8"E	0-2	xi.2007	L.E. Becking
RMNH POR. 3968	#RAJ64/ mol429	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 3969	#RAJ64/ mol430	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 3970	#RAJ64/ mol431	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 3971	#RAJ64/ mol432	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 3972	#RAJ64/ mol433	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 3973	#RAJ39/ mol249	Indonesia	West Papua	Raja Ampat	Fam		rocky shore	00°36'01.5"S	130°45'08"E	0-1	xi.2007	L.E. Becking
RMNH POR. 3974	#RAJ39/ mol250	Indonesia	West Papua	Raja Ampat	Fam		rocky shore	00°36'01.5"S	130°45'08"E	0-1	xi.2007	L.E. Becking
RMNH POR. 3975	#RAJ39/ mol254	Indonesia	West Papua	Raja Ampat	Fam		rocky shore	00°36'01.5"S	130°45'08"E	0-1	xi.2007	L.E. Becking
RMNH POR. 3979	#KKB/mol 779	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4112	#P-TER11	Indonesia	Moluccas		Ternate		reef				xi.2009	N.J. de Voogd

registration number	fieldcode	country	province	region	island	locality	habitat	latitude	longitude	depth	date	collector
RMNH POR. 4113	#P-TER22	Indonesia	Moluccas		Ternate		reef				xi.2009	N.J. de Voogd
RMNH POR. 4489	#KKB/mol721	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4490	#KKB/mol830	Indonesia	East Kalimantan	Berau	Kakaban	Kakaban lake	marine lake	02°08'57.3"N	118°31'26.4"E	0-2	ix.2008	L.E.Becking
RMNH POR. 4491	#BER109/ mol629	Indonesia	East Kalimantan	Berau		lighthouse near Berau river	reef	02°09'49.9"N	118°10'12.8"E	10	16.viii.2008	L.E.Becking
RMNH POR. 4492	#BER111/ mol666	Indonesia	East Kalimantan	Berau	Kakaban	SW Kakaban	reef	02°08'07.5"N	118°30'23.3"E	10	17.viii.2008	N.J. de Voogd
RMNH POR. 4493	#RAJ64/ mol428)	Indonesia	West Papua	Raja Ampat	Waigeo	Teluk Mayabilit	reef	00°18'17.0"S	130°54'15.6"E	10	xii.2007	L.E. Becking
RMNH POR. 4494	#RAJ23/ mol199	Indonesia	West Papua	Raja Ampat	Gam	Ctenophore lake	marine lake	00°27'17.5"S	130°29'33.8"E	0-2	xi.2007	L.E. Becking
ZMA Por. 896		Indonesia	South Sulawesi		SW Salayer	reef N of Pulau Bahuluang	reef	06°27'00"S	120°25'48"E	10-45	30.ix.1984	R.W.M. van Soest (Snellius Expedition II)
ZMA Por. 10495		Seychelles		Mahé	Mahé	SE coast near Pointe Cocos		04°45'00"S	055°32'60"E	35-45	24.xii.1992	R.W.M. van Soest

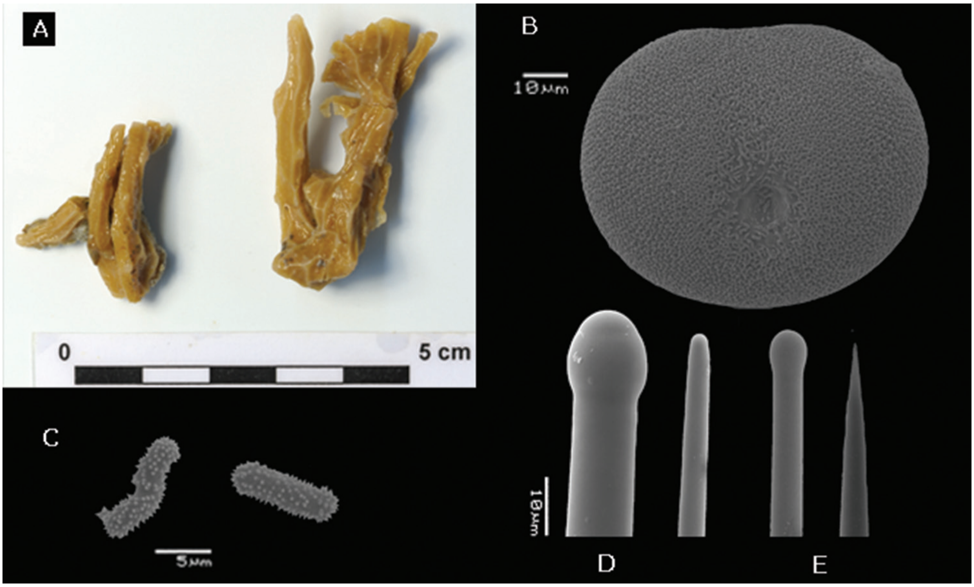

Figure 8. *Placospongia santodomingoae* sp. n. (RMNH POR. 4486). **A** ethanol preserved specimen **B** selenaster **C** large tylostyle (head and blunt end) **D** small tylostyle (head and hastate end) **E** microrhabds.

ua island, Buli Halo anchialine pool, 02°11'16.4"N, 118°37'06.4"E, 0–1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari, #BER128/1156.

Description. Holotype and paratypes are branching and encrusting, size 8cm in length. Total size of specimens *in situ* is hard to establish as parts of the body may be encrusting within cracks. Alcohol preserved and live specimens are hard but slightly compressible. The surface is made up with typical *Placospongia* cortical plates separated by contractible grooves which form a network on the surface. Oscules are present in the grooves. Live color of holotype was dark brown, the paratypes were orange, and these colors were mostly retained after alcohol preservation (Fig. 8A).

Spicules. Holotype (Fig. 8) megascleres large straight tylostyles with blunt point 430-605.5-660 × 13-15.5-20 × 13-18.1-23 µm, small straight tylostyles with sharp point 240-261.3-290 × 5-7.2-8 × 5-8.8-10 µm; microscleres selenasters 80-84.8-90 × 60-67.3-75 µm, acanthose microrhabds 8-12.3-18 × 2.5-2.7-3.5 µm. Range of the paratypes (Table 1) large straight tylostyles with blunt point 430-760 × 13-20 × 15-23 µm, small straight tylostyles with sharp point 190-380 × 5-13 × 8-15 µm, microscleres selenasters 63-93 × 58-75 µm, acanthose microrhabds 5-20 × 2.5-3.5 µm.

Skeleton. The cortical plates consist of densely packed selenasters, microrhabds form a layer over and amidst this selenaster cortex and are also prevalent in choanosomal tissue. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates in radial tracts from the centre core (consisting of densely packed selenaster) to the cortical plates. The sharp ends of the smaller tylostyles can be projected beyond the cortex surface.

Distribution. Presently only recorded from Buli Halo anchialine pool on Maratua island, Berau, East Kalimantan, Indonesia (Fig. 9). For a full description of the pool, see Becking et al. (2011)

Ecology. Depth 0–2m. occurs in anchialine pool, can be exposed to air during low tide and can tolerate great fluctuations in salinity (from 24 to 33 ‰).

Etymology. Named in honor of Nadiezhda K. Santodomingo, the collector of the types, for her years of tireless work in marine science including anchialine research.

Remarks. *Placospongia santodomingoae* sp. n. is similar to *P. carinata,* yet lacks streptasters and has shorter tylostyles. *Placospongia santodomingoae* sp. n. likewise differs from *P. mixta* by the absence of streptasters as well as the absence of spherasters. *Placospongia santodomingoae* sp. n. differs from *P. anthosigma* by the absence of anthosigma, and by having hastate endings of the smaller tylostyles.

Geodia labyrinthica (Kirkpatrick, 1903)

http://species-id.net/wiki/Geodia_labyrinthica

Placospongia labyrinthica Kirkpatrick 1903: Plate V Fig. 1a–b, Plate VI Fig. 1a–f.

Reviewed material. Holotype. BMNH 02.11.16.1, South Africa, East London Coast, 33°06'30"S, 028°11'E.

Spicules. Megascleres styles, oxea; microscleres sterrasters, chiasters

Remarks. This species was originally described as '*Placospongia labyrinthica*', but does not have the characteristic cortical plates of *Placospongia.* The specimen furthermore has sieve pores, sterrasters with star-like plates, euasters, styles and oxea characteristic of the Geodiidae. In the original description, Kirkpatrick (1903) stated "the presence of chiasters is so exceptional that I thought at first that I had to deal with a geodine sponges, but there were no triaenes to be found" and as a result placed this species in the *Placospongia* rather than *Geodia*. Genus transfer to *Geodia* is, however, required as suggested on the World Porifera Database (van Soest et al. 2011).

Identification key for Indo-Pacific species of *Placospongia*

1	Streptasters absent	**2**
–	Streptasters present	**3**
2	Spherules present	***P. melobesioides***
–	Spherules absent	**4**
3	Streptasters have rays with birfurcating ends	***P. carinata***
–	Streptasters have rays with hastate ends, spherasters present	***P. mixta***
4	Spherasters present, microrhabds with short spines spirally places on shaft	***P. anthosigma***
–	Spherasters absent, acanthose microrhabds present	***P. santodomingoae* sp.n.**

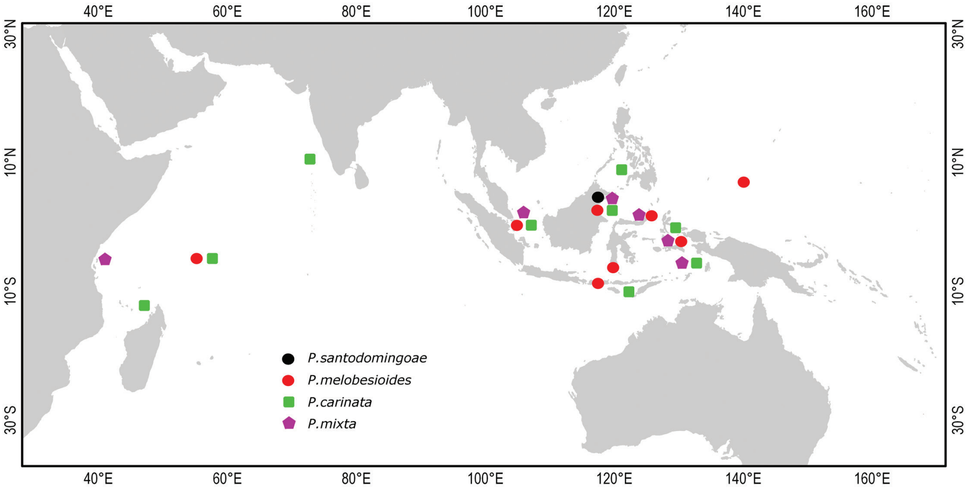

Figure 9. Distribution of *Placospongia* spp. in the Indo-West Pacific. Location of symbols is approximate.

Genetic data analysis

All sequences were submitted to GenBank with accession numbers KC848421 - 41 (Table 5). Final alignments (excluding primers) were obtained for the sponge *Placospongia* of 581 bp for COI with three genetic variants (28 individuals) and 13 polymorphic sites. The three genetic variants correspond to the three species *P. melobesioides, P. mixta,* and *P. carinata* that represent monophyletic groups which are strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 10). There was no intra-specific variation within each species, regardless of geographic locality. The inter-specific *p*-distances ranged between 0.5-2.1% (Table 6). There were 11 substitutions between *P. melobesioides* and *P. carinata,* 12 substitutions between *P. melobesioides* and *P. mixta,* and three substitutions between *P. mixta* and *P. carinata*. The specimens of *P. carinata* and of *P. santodomingoae* sp. n. had identical genotypes for COI. No molecular work could be done on the dried holotype of *Placospongia anthosigma* and fresh material was not available.

Final alignments (excluding primers) of 720 bp were obtained for ITS with 18 genetic variants from the present study (22 individuals). An additional 27 genetic variants from GenBank (for GenBank accession numbers see Fig. 11) were included in the phylogenetic analysis. The ITS sequences represented five clades that were strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 11). These five divergent clades (see Table 7 for uncorrected *p*-distances) correspond to the clades C3, C4, C5, C6, and C9 as presented by the study of Nichols & Barnes (2005). Clade C9 represents specimens of the species *P. melobesioides,* clade C5 *P. mixta,* and clade C4 *P. carinata*. Clades C6 is represented by one specimen from the Solomon Islands (QM317896) and clade C3 by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439); none of the samples that were sequenced in the

Table 5. Specimens of *Placospongia* studied for DNA analysis. Genbank accession numbers provided for sequences of Cytochrome Oxidase I (COI) and internal transcribed spacer region (ITS).

Registration number	Species	COI	ITS
RMNH POR. 4482	*P. carinata*	KC848441	KC848429
RMNH POR. 4483	*P. carinata*	KC848441	KC848427
RMNH POR. 4484	*P. carinata*	KC848441	KC848428
RMNH POR. 4485	*P. carinata*	KC848441	KC848429
ZMA Por. 10727	*P. carinata*	KC848441	-
ZMA Por. 11367	*P. carinata*	KC848441	-
RMNH POR. 2464	*P. melobesioides*	KC848439	-
RMNH POR. 3942	*P. melobesioides*	KC848439	KC848422
RMNH POR. 3976	*P. melobesioides*	KC848439	-
RMNH POR. 4114	*P. melobesioides*	KC848439	KC848426
RMNH POR. 4495	*P. melobesioides*	KC848439	KC848436
RMNH POR. 4496	*P. melobesioides*	KC848439	KC848436
RMNH POR. 4497	*P. melobesioides*	KC848439	KC848437
RMNH POR.3166	*P. melobesioides*	KC848439	KC848422
ZMA Por. 10459	*P. melobesioides*	KC848439	KC848438
RMNH POR. 3158	*P. mixta*	KC848440	KC848421
RMNH POR. 3960	*P. mixta*	KC848440	KC848423
RMNH POR. 3979	*P. mixta*	KC848440	-
RMNH POR. 4113	*P. mixta*	KC848440	KC848425
RMNH POR. 4489	*P. mixta*	KC848440	-
RMNH POR. 4490	*P. mixta*	KC848440	KC848433
RMNH POR. 4491	*P. mixta*	KC848440	KC848433
RMNH POR. 4492	*P. mixta*	KC848440	KC848434
RMNH POR. 4493	*P. mixta*	KC848440	KC848435
RMNH POR. 4494	*P. mixta*	KC848440	KC848435
RMNH POR. 4486	*P. santodomingoae* sp. n.	KC848441	KC848430
RMNH POR. 4487	*P. santodomingoae* sp. n.	KC848441	KC848431
RMNH POR. 4488	*P. santodomingoae* sp. n.	KC848441	KC848432

Table 6. The number of base differences per site from averaging over all Cytochrome Oxidase I (COI) sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal in italic. The analysis involved 30 nucleotide sequences. There was no within-group difference. *Spirastrella* aff. *decumbens* was used as outgroup in the phylogenetic inference (see Fig. 10).

% *p*-distance COI	*P. melobesioides*	*P. mixta*	*P. carinata*	*Spirastrella* aff. *decumbens*
P. melobesioides	*	*0.6*	*0.6*	*1.3*
P. mixta	2.1	*	*0.3*	*1.2*
P. carinata	1.9	0.5	*	*1.3*
Spirastrella aff. *decumbens*	12.2	11.5	11.7	*

present study fell into either C3 or C6 clade. The specimens of *P. santodomingoae* sp. n. represented a separate lineage within the *P. carinata* clade (C4) which was supported by Bayesian inference, but not by maximum likelihood analysis. The *p*-distance between *P. carinata* specimens and the specimens of *P. santodomingoae* sp. n. was 0.6%.

Table 7. The number of base differences per site from averaging over all internal transcribed spacer (ITS) sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal. All positions with less than 5% site coverage were eliminated. Black cursive along the diagonal indicates within-group uncorrected *p*-distance. The analysis involved 73 nucleotide sequences. C9, C5, C6, C4, C3 refer to five clades in the Indo-West Pacific *Placospongia* as presented in Fig. 11.

% *p*-distance ITS	***P .melobesioides***	***P. mixta***	***P. carinata***	***P. santodomingoae* sp. n.**	**C9**	**C5**	**C6**	**C4**	**C3**
P. melobesioides	***0.1***	*1.3*	*1.4*	*1.4*	*0.3*	*1.3*	*1.3*	*1.3*	*1.3*
P. mixta	13.8	***0.7***	*0.9*	*0.9*	*1.2*	*0.2*	*0.5*	*0.9*	*0.9*
P. carinata	14.7	6.3	***0.4***	*0.2*	*1.3*	*0.9*	*0.9*	*0.2*	*0.9*
P. santodomingoae sp. n.	13.2	5.8	0.6	***1.6***	*1.3*	*0.9*	*0.9*	*0.3*	*0.9*
C9	0.9	13.5	14.6	13.6	***0.1***	*1.2*	*1.2*	*1.2*	*1.2*
C5	13.5	0.9	6.6	6.1	12.9	***0.7***	*0.5*	*0.8*	*0.9*
C6	14	2.2	6.4	6.1	13.2	2.2	***0.1***	*0.8*	*0.8*
C4	14.8	6.3	0.5	0.9	14.3	6.3	6	***0.4***	*0.8*
C3	15.2	7.1	6.1	5.9	14.5	6.9	6.3	5.6	***0.9***

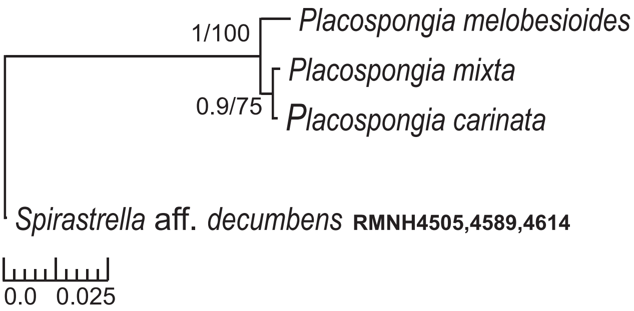

Figure 10. Bayesian/maximum likelihood phylograms of Cytochrome Oxidase I (COI) sequences from Indo-Pacific *Placospongia* spp. See Table 5 for GenBank accession numbers. Only posterior probabilities of >90 and maximum likelihood values of >70 indicated. Scale bar indicates substitutions/site.

Discussion

Different species

In the Indo-West Pacific at least five species of the genus *Placospongia* can be identified based on spicule morphology: *P. anthosigma, P. carinata, P. mixta, P. melobesioides,* and *P. santodomingoae* sp. n.. *Placospongia melobesioides, P. carinata,* and *P. mixta* can be distinguished with the DNA barcode marker (COI) and a nuclear marker (ITS). The species *P. santodomingoae* sp. n. and *P. carinata* have the same sequence of COI. The sequence variation of COI in sponges can be low (e.g. Wörheide 2006, Xavier et al. 2010, Pöppe et al. 2011) and this is also the case in species of *Placospongia*, e.g. only 0.5% nucleotide distance between the species *P. mixta* and *P. carinata.* There is further-

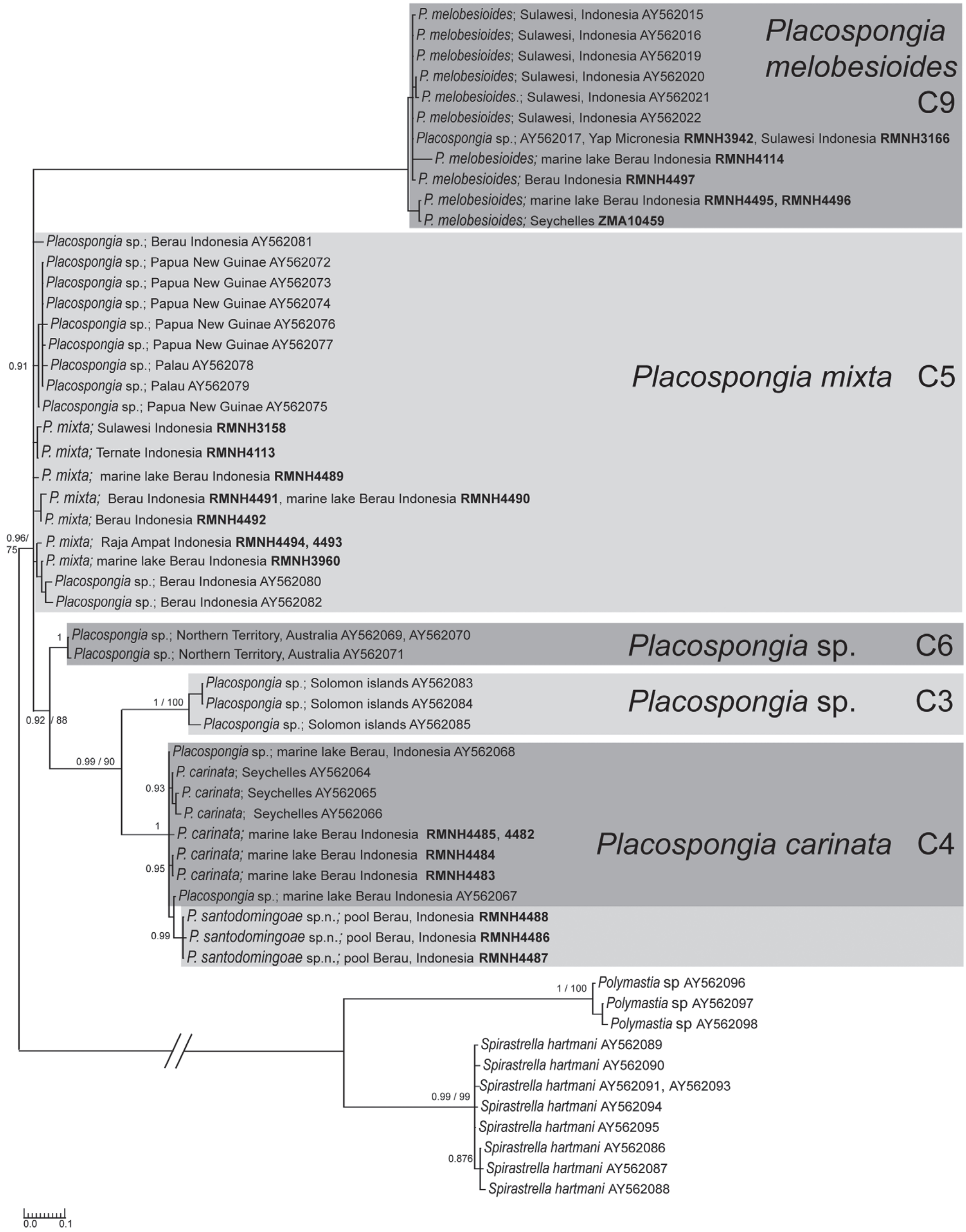

Figure 11. Bayesian/maximum likelihood phylograms of genotypes of the internal transcribed spacer region of nuclear ribosomal operons (ITS) of Indo-Pacific *Placospongia* spp. found in this study and related species from the same genus collected from GenBank. Clades C3, C4, C5, C6 & C9 refer to the clades presented in the study by Nichols & Barnes (2005). Taxon labels are organized as follows: Specimen - Locality - Genbank code or RMNH POR. Number. Only posterior probabilities of >90 and maximum likelihood values of >70 indicated. Scale bar indicate substitutions/site.

more no intraspecific variation in COI within each of the *Placospongia* species, not even between populations at 1000s of km distance from each other (e.g. specimens from the Seychelles are identical with specimens from Indonesia). The phylogenetic inference based on the ITS sequences does show a supported clade of *P. santodomingoae* sp. n. within the clade of *P. carinata* (Fig. 11), though the degree of divergence between the two species is low (0.6%) (Table 7). *Placospongia santodomingoae* sp. n. should, however, be designated as a new species based on the spicule morphology: the absence of a distinguishing spicule type (streptasters) and consistently shorter and thicker tylostyles (maximum 760 × 20 µm) compared to *P. carinata* (maximum 980 × 17.5 µm) are valid arguments to distinguish a separate species within this genus. The specimens of *P. santodomingoae* sp. n. were collected from an anchialine pool. This kind of isolated environment has previously been shown to contain small, rapidly evolving populations, and many rare species across a large spectrum of taxa (e.g. Holthuis 1973, Tomascik & Mah 1994, Dawson & Hamner 2005, Becking et al. 2011, Becking et al. *in press*). The divergence of *P. santodomingoae* sp. n. from *P. carinata* is likely too recent to be expressed in the molecular markers that were used. Other, faster evolving, molecular markers might show a more distinct separation between species, but for the present significant morphometric differences in spicules are reliable characters in separating these sister species.

A molecular phylogeny using the internal transcribed spacer region (ITS) showed that there were five distinct clades within the genus *Placospongia* in the Indo-West Pacific (clades C3, C4, C5, C6 & C9) (Nichols & Barnes, 2005). Nichols & Barnes (2005) indicated that their results presented a conundrum that “specimens collected from Indonesian marine lakes that have been isolated from the surrounding marine environment since the Pleistocene are undifferentiated from individuals collected from the Seychelles indicating that populations from these geographically disparate regions are, or have recently been, connected by gene flow despite the lack of evidence of connectivity between these lakes and nearby reefs.” It is important to note here that the authors did not investigate the spicule morphology of the specimens in their study, while it is in fact the spicules that can largely explain the presented conundrum. In the present study over 30 specimens from the marine lakes Kakaban and Maratua and the adjacent reefs have been reviewed as well as the specimens from the ZMA Por. collection that were used in the Nichols & Barnes (2005) study. Clade C4 represents the material from the Seychelles (ZMA Por.11367) together with the marine lakes and can all be morphologically identified as *P. carinata sensu stricto*. The samples from the lakes and the Seychelles are thus conspecific, but the populations of the two locations are necessarily connected by gene flow. Subsequently clade C9 is *P. melobesioides* (specimens from Indonesia, Miscronesia and the Seychelles) and clade C5 is of *P. mixta* (specimens from Indonesia, Palau and Papua New Guinae). This explains three of the five clades from the Indo-West Pacific and leaves two undetermined: clade C3 represented by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439), and clade C6 represented by one specimen from the Solomon Islands (QM317896). The morphology of these specimens should be further studied in order to correctly identify the species and determine if they may represent morphologically cryptic species.

Natural variation

Each of the five species of the genus *Placospongia* in the Indo-West Pacific can be distinguished based on the composition and morphology of spicules. The external morphology, however, does not allow species distinction. The most common species from the tropical Indo-West Pacific (*P. melobesioides, P. mixta, and P. carinata*) can have both encrusting and branching growth forms displaying a variety of colors from white to dark brown. The only observed consistent pattern was that all the red specimens belonged to *P. mixta,* while all the dark black-brown specimens belonged to *P. melobesioides.* These two colors may be useful for field identifications, yet both species can also display the range of other colors (white, cream, beige, light brown) as well. The density of canals/ridges (or size of cortical plates) appears to be related to environment as this is higher in specimens from high sediment locations such as the marine lakes than in specimens from the reefs (Fig. 1, 2). Within each species there is also some natural variation in the range of tylostyle length and spicule morphology. The streptaster morphology varies within species and even within individuals. Within one individual the number of rays can vary from 4-10 (Figs 3, 4) and between individuals the decoration and size of spines can be diverse. For example the streptasters of *P. carinata* specimens from Haji Buang marine lake are micro-acanthose while the specimens from other locations are not. Spherasters are always present and abundant in *P. mixta* and *P. anthosigma,* but are in low abundances or absent in *P. melobesioides*, as has been indicated previously by Vosmaer & Vernhout (1902). In *P. carinata* and *P. santodomingoae* sp. n. spherasters are always absent.

Ecology and distribution

P. melobesioides and *P. mixta* are common in the reef environment. Most of the collected material from the reefs in Indonesia were one of these two species. *P. carinata* appears to be rare in the reefs, in Indonesia at least, while it is highly abundant in the marine lakes Haji Buang and Kakaban in East Kalimantan, Indonesia. *Placospongia santodomingoae* sp. n. is restricted to an anchialine pool. *Placospongia anthosigma* was not found in any of the examined collections from the tropical Western Pacific, this species is restricted to more temperate and deeper waters. *Placospongia melobesioides* is indicated in the Systema Porifera to have a distribution from the Indo-West Pacific to the Tropical Atlantic (Rützler 2002). Both *P. melobesioides* and *P. carinata* have been recorded from the Atlantic (e.g. de Laubenfels 1936, Hechtel 1976, Coelho and Mello-Leitão 1978, Pulitzer-Finali 1986, González-Farías 1989), which would imply that these are pantropical species. Recent molecular and more detailed morphological studies have, however, shown that many cosmopolitan sponge species are in fact species complexes either delineated by morphology or molecules (e.g. Reveilleud et al. 2010, Xavier et al. 2010). Van Soest (2009) has indicated that there are at least five species of *Placospongia* in the Caribbean that are morphologically different from the holotypes of *P. melobesioides* and *P. carinata.* Rua et al. (2006) and Nichols and Barnes (2005), furthermore, show that there are distinct lineages in the Caribbean and Western Pacific, that are not shared between the two regions and that most likely represent undescribed species in the Caribbean. Considering these results as well as the large geographic distance between the Caribbean and the type localities of *P. melobesioides* and *P. carinata* (both Indo-West Pacific), it is highly unlikely that these species occur in the Tropical Atlantic. Further revision of the Atlantic and Eastern Pacific material will shed more light on this issue.

Future biodiversity surveys and species checklists both in the Atlantic as well as in the Pacific are advised to check the spicule morphology of *Placospongia* specimens in order to identify species, as the external morphology and color will not give an indication to the number of species. The different *Placospongia* spp. can occupy the same type of habitats in the tropics. An example of such sympatry is represented in Kakaban lake where in the 4 km^2 area of the marine lake the three common tropical species of *Placospongia* co-exist side by side. Neglecting to review the spicule morphology would mean possibly missing the true diversity of species that are present in the location of study.

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References

Becking LE, Cleary DFR, de Voogd NJ, Renema W, de Beer M, van Soest RWM, Hoeksema BW (2006) Beta diversity of tropical marine benthic assemblages in the Spermonde archipelago, Indonesia. Marine Ecology 27: 76–88. doi: 10.1111/j.1439-0485.2005.00051.x

Becking LE, Renema W, Santodomingo NK, Hoeksema BW, Tuti Y, de Voogd NJ (2011) Recently discovered landlocked basins in Indonesia reveal high habitat diversity in anchialine systems. Hydrobiologia 677: 89–105. doi: 10.1007/s10750-011-0742-0

Becking LE, Cleary DF, de Voogd NJ (in press) Sponge species composition, abundance and cover in marine lakes and coastal mangroves of Berau, Indonesia. Marine Ecology Progress Series.

Bowerbank JS (1858) On the Anatomy and Physiology of the Spongiadae. Part I. On the Spicula. Philosophical Transactions of the Royal Society 148(2): 279–332.

Bowerbank JS (1874) Contributions to a General History of the Spongiadae. Part VI. Proceedings of the Zoological Society of London 1874: 298–305, pls XLVI-XLVII.

Burton M (1959) Sponges. In: Scientific Reports. John Murray Expedition, 1933–34. 10 (5).

British Museum (Natural History), London, 151–281.

Coelho EP, Mello-Leitão A (1978) *Placospongia carinata* e sua ocorrência em costas brasileiras. Avulso Departamento de Zoologia, Universidade Universidade Federal do Rio de Janeiro, Rio de Janeiro 29: 1–5.

Dawson MN, Hamner WM (2005) Rapid evolutionary radiation of marine zooplankton in peripheral environments. Proceedings of the National Academy of Science 102: 9235–9240. doi: 10.1073/pnas.0503635102

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology Biotechnology 3: 294–299.

González-Farías F (1989) Estudio al microscopio electrónico de barrido de la estructura esquelética de la esponja Placospongia melobesioides Gray. Anales del Instituto de Ciencias del Mar y Limnologia Universidad Nacional Autónoma de México, 16 (1): 81–90.

Gray JE (1867) On *Placospongia*, a new generic form of Spongiadae in the British Museum. Proceedings of the Zoological Society of London 1867 (1): 127–129.

Hechtel GJ (1976) Zoogeography of Brazilian Marine Demospongiae. In: Harrison FW, Cowden RR (Eds) Aspects of Sponge Biology. Academic Press, New York & London, 237–260.

Holthuis LB (1973) Caridean Shrimps found in land-locked saltwater pools at four Indo-West Pacific localities (Sinai Peninsula, Funafuti Atoll, Maui and Hawaii Islands), with the description of one new genus and four new species. Zoologische Verhandelingen, Leiden 128: 1–48.

Hooper JNA, Wiedenmayer F (1994) Porifera. In: Wells A (Ed) Zoological Catalogue of Australia Vol. 12. CSIRO, Melbourne, 1–621.

Hooper JNA, Kennedy JA, van Soest RWM (2000) Annotated checklist of sponges (Porifera) of the South China Sea region. Raffles Bulletin Zoological Supplement 8: 125–207.

Hooper JNA, Van Soest RWM (2002) Systema Porifera. A guide to the classification of sponges. Volume 1. Kluwer Academic/ Plenum Publishers: New York, Boston, Dordrecht, London, Moscow. doi: 10.1007/978-1-4615-0747-5

Huelsenbeck JP, Ronquist F (2001) Mrbayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. doi: 10.1093/bioinformatics/17.8.754

Kieschnick O (1896) Silicispongiae von Ternate nach den Sammlungen von Herrn Prof. Dr. W. Kükenthal. Zoologischer Anzeiger 19(520): 526–534.

Kirkpatrick R (1903) Descriptions of South African Sponges. Part III. Marine Investigations in South Africa 2(14): 233–264.

de Laubenfels MW (1936) A discussion of the sponge fauna of the Dry Tortugas in particular and the West Indies in general, with material for a revision of the families and orders of the Porifera. Carnegie Institute of Washington (Tortugas Laboratory Paper N° 467) 30: 1–225.

Lévi C (1956) Spongiaires des côtes de Madagascar. Mémoires de l'Institut scientifique de Madagascar (A) 10: 1–23.

Meyer CP, Geller JB, Paulay G (2005) Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. Evolution 59: 113–125.

Nichols SA, Barnes PAG (2005) A molecular phylogeny and historical biogeography of the marine sponge genus Placospongia (Phylum Porifera) indicate low dispersal capabilities and widespread crypsis. Journal of Experimental Marine Biology and Ecology 323: 1–15. doi: 10.1016/j.jembe.2005.02.012

Pöppe J, Sutcliffe P, Hooper JNA, Wörheide G, Erpenbeck D (2011) CO I Barcoding Reveals New Clades and Radiation Patterns of Indo-Pacific Sponges of the Family Irciniidae (Demospongiae: Dictyoceratida). PlosOne 5(4): e9950. doi: 10.1371/journal.pone.0009950

Posada D (2008) Jmodeltest: Phylogenetic model averaging. Molecular Phylogenetics & Evolution 25:1253–1256.

Pulitzer-Finali G (1986) A collection of West Indian Demospongiae (Porifera). In appendix, a list of the Demospongiae hitherto recorded from the West Indies. Annali del Museo Civico di Storia Naturale Giacomo Doria 86: 65–216.

Pulitzer-Finali G (1993) A collection of marine sponges from East Africa. Annales Museo Civico Storia Naturale "Giacomo Doria" 89: 247–350.

Rambaut A, Drummond AJ (2007) Tracer v1.4, available from http://beast.Bio.Ed.Ac.Uk/tracer

Reveillaud J, Remerie T, van Soest R, Erpenbeck D, Cardenas P, Derycke S, Xavier J, Rigaux A, Vanreusel A (2010) Species boundaries and phylogenetic relationships between Atlanto-Mediterranean shallow-water and deep-sea coral associated *Hexadella* species (Porifera, Ianthellidae). Molecular Phylogenetics & Evolution 56: 104–114. doi: 10.1016/j.ympev.2010.03.034

Rua CPJ, Mattos A, Solé-Cava A (2006) Cryptic speciation and correspondence between spiculation and molecular markers in *Placospongia*. In: Book of Abstracts 7th International Sponge Symposium, Biodiversity, Innovation, Sustainability. Museu Nacional, Rio de Janeiro, 197.

Rützler K (2002) Family Placospongiidae Gray, 1867. In: Hooper JNA, van Soest RW M (Ed.) Systema Porifera. A guide to the classification of sponges. 1 (Kluwer Academic/Plenum Publishers: New York, Boston, Dordrecht, London, Moscow), 196–200. doi: 10.1007/978-1-4615-0747-5_21

van Soest RWM (2009) New sciophilous sponges from the Caribbean (Porifera: Demospongiae). Zootaxa 2107: 1–40.

van Soest RWM, Beglinger EJ (2008) Tetractinellid and hadromerid sponges of the Sultanate of Oman. Zoologische mededelingen Leiden 82 (53): 749–79.

van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, de Voogd NJ, Alvarez de Glasby B, Hajdu E, Pisera AB, Manconi R, Schoenberg C, Janussen D, Tabachnick KR, Klautau M, Picton B, Kelly M (2011) World Porifera database. http://www.marinespecies.org/porifera [accessed 31.I.2011]

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology & Evolution 28: 2731–2739. doi: 10.1093/molbev/msr121

Tanita S, Hoshino T (1989) The Demospongiae of Sagami Bay. Biological Laboratory, Imperial Household Japan, 197pp.

Thiele J (1900) Kieselschwämme von Ternate. I. Abhandlungen herausgegeben von der Senckenbergischen naturforschenden Gesellschaft Frankfurt 25: 19–80.

Tomascik T, Mah AJ (1994) The ecology of ”*Halimeda* lagoon”: an achialine lagoon of a raised atoll, Kakaban Island, East Kalimantan, Indonesia. Tropical Biodiversity 2: 385–399.

de Voogd NJ, Cleary DFR (2008) An analysis of sponge biodiversity and distribution at three taxonomic levels in the Thousand Islands/Jakarta Bay reef complex, West-Java,Indonesia. Marine Ecology 29: 205–215. doi: 10.1111/j.1439-0485.2008.00238.x

de Voogd NJ, Becking LE, Cleary DFR (2009) Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia. Marine Ecology Progress Series (396): 169–180. doi: 10.3354/meps08349

Vosmaer GCJ, Vernhout JH (1902) The Porifera of the Siboga-Expedition. I. The genus *Placospongia*. *In*: Weber M (Ed) Siboga-Expeditie. Uitkomsten op zoologisch, botanisch, oceanographisch en geologisch gebied verzameld in Nederlandsch Oost-lndië 1899–1900aan boord H.M. 'Siboga' onder commando van Luitenant ter Zee 1e kl. G.F. Tydeman. 9 (Monographie VIa) Brill EJ, Leiden, 1–17.

Wörheide G (2006) Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge *Astrosclera willeyana* across the Indo-Pacific. Marine Biology 148(5): 907–912. doi: 10.1007/s00227-005-0134-y

Xavier JR, Rachello-Dolmen PG, Parra-Velandia F, Schoenberg CHL, Breeuwer JAJ, van Soest RWM (2010) Molecular evidence of cryptic speciation in the "cosmopolitan" excavating sponge *Cliona celata* (Porifera, Clionaidae). Molecular Phylogenetics & Evolution 56: 13–20. doi: 10.1016/j.ympev.2010.03.030

Xia X, Xie Z (2001) DAMBE: Software Package for Data Analysis in Molecular Biology and Evolution. Journal of Heredity 92(4): 371–373. doi: 10.1093/jhered/92.4.371

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A relict lineage and new species of green palm-pitviper (Squamata, Viperidae, *Bothriechis*) from the Chortís Highlands of Mesoamerica

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Abstract

A new species of palm-pitviper of the genus *Bothriechis* is described from Refugio de Vida Silvestre Texíguat in northern Honduras. The new species differs from congeners by having 19 dorsal scale rows at midbody, a bright green dorsal coloration in adults, the prelacunal scale fused to the second supralabial, and in representing a northern lineage that is sister to *B. lateralis*, which is distributed in Costa Rica and western Panama and is isolated from the new taxon by the Nicaraguan Depression. This represents the 15th endemic species occurring in Refugio de Vida Silvestre Texíguat, one of the richest herpetofaunal sites in Honduras, itself being the country with the highest degree of herpetofaunal endemism in Central America. We name this new species in honor of a Honduran conservationist slain in fighting against illegal logging, highlighting the sacrifices of rural activists in battling these issues and the critical importance of conservation in these areas.

Resumen

Una nueva especie de tamagás verde del género *Bothriechis* se describe del Refugio de Vida Silvestre Texíguat en el norte de Honduras. La nueva especie difiere de sus congéneres por tener filas de 19 escamas dorsales en la mitad del cuerpo, una brillante coloración verde en la porción dorsal del cuerpo en adultos, la escama prelacunal fusionada con la segunda supralabial, y en representación del linaje del norte que es clado hermano de *B. lateralis*, la cual se distribuye en Costa Rica y el Occidente de Panamá y está aislada del nuevo taxón por la Depresión de Nicaragua. Representa la decimoquinta especie endémica encontrada en el Refugio de Vida Silvestre Texíguat, uno de los lugares más ricos de herpetofauna en Honduras, el país con el más alto grado de endemismo de la herpetofauna en Centroamérica. Nombramos esta nueva especie en honor a un conservacionista Hondureño asesinado en contienda contra la tala ilegal, destacando los sacrificios de los activistas rurales luchando frente a estos problemas y la importancia crítica de la conservación en estas áreas.

Keywords

Bothriechis guifarroi sp. n., *Bothriechis lateralis*, *Bothriechis marchi*, Central America, conservation, cryptic species, endemic, Honduras, Pico Bonito National Park, Texíguat Wildlife Refuge

Palabras claves

Bothriechis guifarroi sp. n., *Bothriechis lateralis*, *Bothriechis marchi*, Centroamérica, conservación, endemismo, especies crípticas, Honduras, Parque Nacional Pico Bonito, Refugio de Vida Silvestre Texíguat

Introduction

In the past decade, a steady stream of taxonomic discoveries have come out of the Chortís Highlands of Mesoamerica, a biogeographic region found to the south and east of the tectonic boundary between the Chortís and Mayan Blocks and north of the Nicaraguan Depression (Townsend 2011, Townsend et al. 2011). Fifty new species of amphibians and reptiles have been described from the region's montane forests since 2000 (Cadle 2012, Cadle and Savage 2012, McCranie and Hedges 2012, Rovito et al. 2012), with literally dozens more awaiting description (Townsend 2011).

Our knowledge of the taxonomic diversity of Mesoamerican pitvipers has also greatly increased since the turn of the century (e.g. Campbell and Flores-Villela 2008, Jadin et al. 2011). Three species of endemic pitvipers have been described from the Chortís Highlands since 2000: *Atropoides indomitus* Smith & Ferrari-Castro 2008, *Bothriechis thalassinus* Campbell & Smith 2000, and *Cerrophidion wilsoni* Jadin, Townsend, Castoe & Campbell 2012. Two of these three taxa, *B. thalassinus* and *C. wilsoni*, had previously been concealed within more widespread taxa only to be revealed by more focused sampling and phylogenetic analyses.

The green palm-pitvipers (genus *Bothriechis*) of Mesoamerica have long been a source of taxonomic uncertainty and confusion (Campbell and Lamar 2004). Ambiguities among type specimens and localities, the imprecise provenance of many available specimens, disjunct distributions limited to fragmented highland forests, and misleading external morphology have all contributed to a lack of taxonomic resolution among populations currently assigned to two species from the Chortís Highlands; *Bothriechis marchi* (Barbour and Loveridge 1929) and *B. thalassinus*. As currently understood, these two taxa inhabit a number of disjunct localities in the Chortís Highlands (Campbell and Lamar 2004, McCranie 2011a). Available molecular data for these two taxa are limited to a single sample assigned to each nominal form, which indicate the two are sister species nested within a Nuclear Central American clade (also including *B. aurifer*, *B. bicolor*, and *B. rowleyi*) that is, in turn, sister to the highland *Bothriechis* (i.e. *B. lateralis* and *B. nigroviridis*) found in lower Central America (Taggart et al. 2001, Castoe and Parkinson 2006, Castoe et al. 2009).

Bothriechis marchi sensu lato is known from localities in the Cordillera Nombre de Dios, Cordillera de Merendón, and Sierra de Sulaco of Honduras and adjacent areas of Guatemala, with *B. thalassinus* being found in the Cordillera de Merendón of Guatemala and Honduras, Cerro Santa Bárbara and nearby highland forests in Honduras, Cerro del Mono in eastern Guatemala, and the highlands around the El Salvador-Guatemala-Honduras border area (Campbell and Lamar 2004; McCranie 2011a). Both of these taxa include a number of allopatric highland populations that have not been assessed using phylogenetic methods. Of interest here are populations from the Cordillera Nombre de Dios, found within and around Refugio de Vida Silvestre Texíguat and Parque Nacional Pico Bonito. These two cloud forest reserves are each recognized for their diverse endemic herpetofauna (McCranie and Castañeda 2005; Townsend et al. 2012) and are taxonomically and biogeographically distinctive from that of the northern Cordillera de Merendón, which includes the vicinity of the type locality of *B. marchi* in the Sierra de Omoa (Townsend and Wilson 2008).

Two expeditions in 2010 provided the first herpetofaunal inventory of the extensively forested windward portions of Refugio de Vida Silvestre Texíguat, one of the most endemism-rich highland forests in Mesoamerica (Townsend et al. 2012). During two visits to the windward side of Refugio de Vida Silvestre Texíguat in June and July 2010, we collected a series of arboreal pitvipers representative of those assigned to *B. marchi*. Phylogenetic analyses revealed that the population from Refugio de Vida Silvestre Texíguat is not conspecific with the nominal taxon *B. marchi*, nor are they part of the Nuclear Central American clade containing *B. marchi* and *B. thalassinus*. Remarkably, this population is shown to represent a relict northern lineage that is most closely related to *B. lateralis* from Costa Rica and western Panama. We herein describe the Refugio de Vida Silvestre Texíguat population of *Bothriechis* as a new taxon, and discuss the implications for systematics, biogeography, and conservation.

Materials and methods

Field-based sampling

The type series was collected during sampling in the vicinity of La Liberación (15.53°N, 87.29°W; camp established at 1,030 m elevation) during 10–21 June (11 participants; 1,320 person-hours sampling) and 26 July–2 August 2010 (13 participants; 880 person hours). Tissue samples were preserved in SED buffer (20% DMSO, 0.25 M EDTA, pH 7.5, NaCl saturated; Seutin et al. 1991, Williams 2007) and whole specimens in 10% formalin and later transferred to 70% ethanol. Specimens were deposited in the Carnegie Museum of Natural History (CM), Museum of Vertebrate Zoology, University of California Berkeley (MVZ), National Museum of Natural History, Smithsonian Institution (USNM), and Amphibian and Reptile Diversity Research Center, University of Texas at Arlington (UTA).

DNA extraction, amplification, and sequencing

Genomic DNA was isolated from muscle tissue taken from eleven specimens of *Bothriechis* using a Qiagen DNeasy extraction kit and protocol. Four mitochondrial gene fragments (NADH dehydrogenase subunit 4 (ND4), cytochrome b (cyt *b*), 12S rRNA, and 16S rRNA) were independently PCR-amplified as described in multiple studies (Knight and Mindell 1993; Arévalo et al. 1994; Parkinson et al. 1997; Parkinson et al. 2002) using Promega GoTaq® Green master mix, the primer pairs ND4 + LEU, Gludg + AtrCB3, L1091 + 12E, and 16SF + 16SR, and annealing temperatures 48°C, 48°C, 50°C, and 45°C, respectively. Sequencing was performed in both forward and reverse directions using the PCR primers on a Beckman Coulter automated capillary sequencer, and sequence chromatographs were edited using Sequencher 4.2. Sequences for each gene were aligned separately, first automatically using the program MUSCLE (Edgar 2004), and then manually rechecked using Se-Al v2.0a11. Gaps in alignments were treated as missing data. No internal stop codons were found in the two protein-coding gene fragments. Novel sequences from this study were deposited in GenBank (KC847255–289).

Previously published sequences of *Bothriechis* were downloaded from GenBank and combined with new sequence data generated in this study (Table 1). Representatives of two Mesoamerican genera from the diverse sister clade to *Bothriechis* (which contains *Atropoides* [Mesoamerica], *Bothriopsis* [South America], *Bothrocophias* [South America], *Bothrops* [Mexico to South America], *Cerrophidion* (Mesoamerica], and *Porthidium* [Mexico to South America]) were selected for use as outgroups to root our *Bothriechis* phylogeny (Castoe et al. 2005, 2009; Daza et al. 2010): *Atropoides mexicanus* and *Cerrophidion wilsoni*, both of which are sympatric with *Bothriechis* in Refugio de Vida Silvestre Texíguat (Townsend et al. 2012).

Table 1. Taxa, vouchers, locality data, and GenBank accession numbers for sequences used in this study. Novel sequences from this study are indicated in boldface; country codes used as follows: CR = Costa Rica; EC = Ecuador; GT = Guatemala; HN = Honduras; MX = Mexico; NI = Nicaragua.

Taxon	**Locality**	**Voucher**	**GenBank Accession Numbers**			
			ND4	**cyt *b***	**12S**	**16S**
Atropoides mexicanus	HN: Atlántida: Texíguat	USNM 578906	**KC847289**	**KC847271**	**KC847268**	**KC847255**
Bothriechis aurifer	GT:	UTA R-35031	DQ305483	DQ305466	DQ305425	DQ305448
Bothriechis bicolor	GT:	UTA R-34156	DQ305484	DQ305467	DQ305426	DQ305449
Bothriechis guifarroi	HN: Atlántida: Texíguat	CM 156870	—	**KC847280**	—	**KC847260**
	HN: Atlántida: Texíguat	MVZ 269305	—	**KC847279**	—	**KC847258**
	HN: Atlántida: Texíguat	USNM 579873	**KC847286**	**KC847274**	**KC847267**	**KC847262**
	HN: Atlántida: Texíguat	USNM 579874	**KC847288**	**KC847282**	**KC847266**	**KC847263**
	HN: Atlántida: Texíguat	USNM 579875	**KC847287**	**KC847281**	**KC847265**	**KC847264**
	HN: Atlántida: Texíguat	USNM 579876	—	**KC847276**	—	—
	HN: Atlántida: Texíguat	USNM 579877	—	**KC847278**	—	—
	HN: Atlántida: Texíguat	USNM 579878	—	**KC847277**	—	**KC847261**
	HN: Atlántida: Texíguat	UTA R-60303	—	**KC847275**	—	**KC847259**
Bothriechis lateralis	CR: Acosta	MZUCR-11155	U41873	AY223588	AF057211	AF057258
Bothriechis marchi	GT: Zacapa: Cerro del Mono	UTA R-52959	DQ305486	DQ305469	DQ305428	DQ305451
	HN: Cortés: Sierra de Omoa	MVZ 263604	—	**KC847283**	—	—
Bothriechis nigroviridis	CR: San Gerondo de Dota	MZUCR-11151	AY223635	AY223589	AF057212	AF057259
Bothriechis rowleyi	MX: Cerro Baúl	JAC 13295	DQ305485	DQ305468	DQ305427	DQ305450
Bothriechis schlegelii	CR: Cariblanco de Sarapiquí	MZUCR-11149	AY223636	AY223590	AF057213	AF057260
	EC: Pichincha	FHGO Live coll	AF292611	AF292573	—	—
	HN: Cortés: Yojoa	UF 157577	**KC847285**	**KC847272**	**KC847270**	**KC847257**
	NI: Jinotega: Bosawas	UF 166874	**KC847284**	**KC847273**	**KC847269**	**KC847256**
Bothriechis supercilliaris	CR: San Vito	—	DQ305487	DQ305470	DQ305429	DQ305452
Bothriechis thalassinus	GT: Zacapa	UTA R-52958	DQ305482	DQ305465	DQ305424	DQ305447
Cerrophidion wilsoni	HN: Olancho: Botaderos	UTA R-52953	JQ724172	JQ724159	JQ724146	JQ627132

Phylogenetic analyses

Bayesian inference (BI) and maximum likelihood (ML) were implemented to reconstruct phylogenies for the *Bothriechis* ingroup taxa. To identify appropriate models of nucleotide substitution for both analyses, we used the program MrModeltest v2.2 (Nylander 2004), run in PAUP* v4.0b10 (Swofford 2002). We used Akaike information criterion (AIC) to select the best-fit models, as estimated by MrModeltest (Table 2). The four gene fragments were concatenated (2,263 total bp), and this combined dataset was partitioned by gene and codon position (for cyt-b and ND4), resulting in a total of eight partitions as was shown to be justified in analysis of a similar dataset that included these four fragments from these species (Castoe and Parkinson 2006). Stems and loops were not partitioned separately due to a lack of informative characters.

Phylogenetic analyses using BI were conducted with MrBayes v3.0b4 (Ronquist and Huelsenbeck 2003). Two simultaneous BI runs were conducted (with the default Markov chain Monte Carlo [MCMC] settings), and run for a total of 5.0×10^6 generations per run, sampling trees and parameters every 100 generations. We used PSRF values (output by MrBayes), together with plots of cold chain likelihood values and parameter estimates visualized in Tracer v1.5.4 (Rambaut and Drummond 2009), to confirm stationarity and convergence of MCMC runs. Based on this evaluation, the first 1.5×10^5 generations from each run were discarded as burn-in.

Phylogenetic relationships were inferred using ML as implemented in RAxML 7.2.8 (Stamatakis 2006, Stamatakis et al. 2008), using the same partitioning scheme described above for BI. Tree support was assessed using the rapid-bootstrapping algorithm with 1000 non-parametric bootstraps; all ML estimates and tests were run under the GTRCAT model, as models available for use in RAxML are limited to variations of the general time-reversible (GTR) model of nucleotide substitution.

Morphological data collection

We examined 34 preserved specimens of *Bothriechis* for this study (Appendix). Definitions of scale counts and morphological features follow Campbell and Lamar (2004) and bilateral characters are reported as right/left. Unsexed juvenile specimens were considered separately from adult males and females. For the holotype, we dissected and removed the partially everted hemipenis at the base. We then filled the hemipenis with warm water using a blunt-tipped syringe needle in order to attempt full eversion. We then removed the water and injected hot petroleum jelly with blue wax-dye until near maximum expansion was achieved. Finally, we tied the hemipenes and stored them in 70% ethanol. This procedure is modified from that of Myers and Cadle (2003) and Zaher and Prudente (2003) and is further described and illustrated in Smith and Ferrari-Castro (2008) and Jadin and Smith (2010). Hemipenial terminology follows

Table 2. Results from *a priori* model selections based on *Akaike* information criterion (AIC) conducted in MrModeltest 2.2 (Nylander, 2004) for partitions of the dataset.

Partition	Total characters	Parsimony-informative characters	Best-fit model
ND4 1st pos	222	39	GTR+Γ
ND4 2nd pos	222	9	GTR+I
ND4 3rd pos	222	128	GTR+Γ
Cyt *b* 1st pos	231	33	GTR+Γ
Cyt *b* 2nd pos	231	12	HKY+I
Cyt *b* 3rd pos	231	134	GTR+I+Γ
12S	409	60	GTR+Γ
16S	495	39	GTR+I

Dowling and Savage (1960), Keogh (1999), and Savage (2002). Comparative morphological data on related species was taken primarily from Campbell and Lamar (2004) and Solórzano (2004). Color names and codes used in descriptions of coloration in life are from Köhler (2012); color notes in life were derived from a series of photographs of the holotype and paratypes.

Results

Bayesian and Maximum Likelihood phylogenetic analyses produced congruent topological results. Our phylogeny (Fig. 1) is generally congruent with those of Castoe et al. (2009) and Daza et al. (2010), recovering two clades of nominal *Bothriechis schlegelii* (one Mesoamerican, one from Ecuador) rendered paraphyletic with respect to *B. supraciliaris*, and showing strong support for a *B. marchi–B. thalassinus* clade and a *B. aurifer–B. rowleyi* clade, together forming a *B. aurifer–B. bicolor–B. marchi–B. rowleyi–B. thalassinus* clade that geographically corresponds to Nuclear Central America (Fig. 1). Both of our analyses recovered a weakly supported clade that includes the Costa Rica/Panama taxa *B. lateralis* and *B. nigroviridis*, along with the *Bothriechis* population from Refugio de Vida Silvestre Texíguat, Honduras. Within this primarily southern clade, nine samples from Refugio de Vida Silvestre Texíguat show virtually no genetic divergence from one another, and form a monophyletic group with a well-supported (PP = 1.0; bs = 100) sister clade to *B. lateralis* from Costa Rica and western Panama (Fig. 1).

Based on the phylogenetic results, we examined morphological variation among populations of *Bothriechis marchi sensu lato*, which confirm the evolutionary and taxonomic distinctiveness of the Texíguat population as well as its apparent morphological affinity with a specimen from Parque Nacional Pico Bonito, approximately 75 km to the east of Refugio de Vida Silvestre Texíguat. We present the following description of this relict northern lineage as a new species.

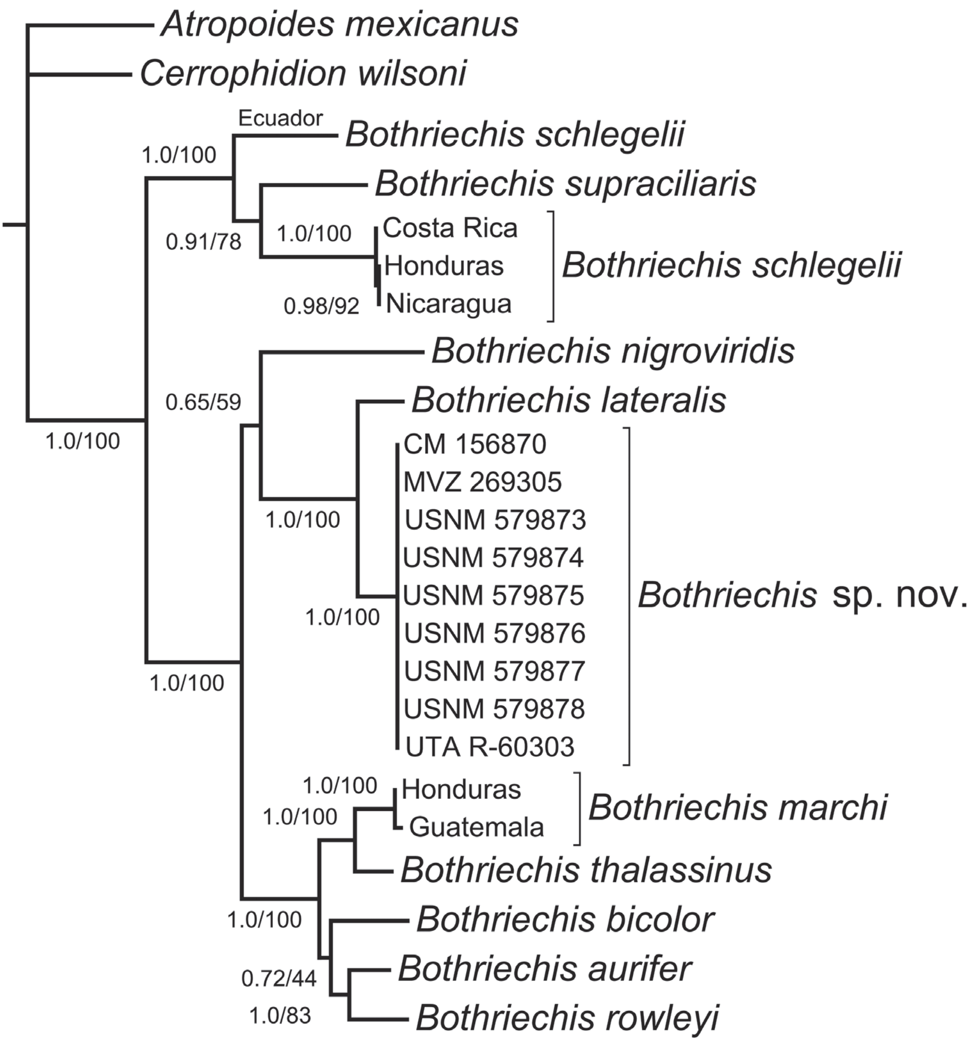

Figure 1. Phylogeny of palm-pitvipers (genus *Bothriechis*), showing strong support for a species-level clade of the Texíguat population (*Bothriechis* sp. n.) that is sister to *B. lateralis*. The tree was estimated from a Bayesian 50% majority-rule consensus composed from a concatenated mitochondrial dataset (ND4, cyt *b*, 12S, and 16S; total of 2263 bp). Numbers at nodes represent values of Bayesian posterior probabilities (PP, left) and Maximum Likelihood bootstraps (BS, right). Nodes supported by ≥ 95% PP and ≥ 70 BS are considered highly supported.

Systematics

Bothriechis guifarroi **sp. n.**
urn:lsid:zoobank.org:act:1FC0661F-B08B-4D0D-85CC-DAEAB502D0DB
http://species-id.net/wiki/Bothriechis_guifarroi
Figs 2–3, 5–6

Bothrops nigroviridis (in part): Meyer 1969: 420.
Bothriechis marchi (in part): Campbell 1982: 381.
Bothrops marchii (in part): Wilson and Meyer 1985: 120.

Holotype. UTA R-60303 (Figs 2, 3), an adult male from La Liberacíon (Fig. 4A,C), 15.5302°N, 87.2939°W (DD), 1,015 m elevation, Refugio de Vida Silvestre Texíguat, Departamento de Atlántida, Honduras, collected 25 July 2010 by the field team of E. Aguilar, A. Contreras, L. Gray, L.A. Herrera-B., M. Medina-Flores, A. Portillo, A. Stubbs, and J. H. Townsend. Original field number JHT 3243. Genbank accession numbers: 16S (KC847259), cyt *b* (KC847275).

Paratypes (8): HONDURAS: Departamento de Atlántida: Refugio de Vida Silvestre Texíguat: adult female (USNM 579875) collected 19 June 2010, two adult females (USNM 579876–77) collected 29 July 2010, and two unsexed neonates collected 18 June 2010 (USNM 579873–74), all from Cerro El Chino (Fig. 4B), 15.5225°N, 87.2802°W (DD), 1,360–1,450 m elevation, southeast of La Liberación. Two males (CM 156870 and MVZ 269305) collected 28 July 2010 from a ridge-top trail above La Liberación, 15.5418°N, 87.2891°W (DD), 1,290 m elevation. One male (USNM 579878) collected 30 July 2010 from La Liberación (Fig. 4A, C), 15.5302°N, 87.2939°W (DD), 1,015 m elevation.

Referred specimens (4). HONDURAS: Departamento de Atlántida: AMNH 46949 from "Tela," collected sometime before April 1932; USNM 319942 from Quebrada de Oro, Parque Nacional Pico Bonito. Departamento de Yoro: Refugio de Vida Silvestre Texíguat: USNM 337488–89 from 2.5 airline km north-northeast of La Fortuna. See Remarks.

Definition. *Bothriechis guifarroi* is distinguished from all nine congeners by the following combination of features: dorsal scales in 19-19-15 rows; ventrals in males 162–166 (163.8), in females 158–166 (164.0), 162–166 (164.0) in neonates; subcaudals in males 60–68 (63.0), in females 60–63 (61.0), 62–68 (65.0) in neonates; intersupraoculars (3–7); superciliary scales absent; prelacunal scale fused to second supralabial on both sides; two known color patterns in juveniles, one brown (with a pale paraventral stripe and a series of short darker dorsal blotches and a dark brown postocular stripe bordered by yellow on its lower edge) and the other green (with a series of pale blue blotches and a deep blue postocular stripe bordered by pale blue on its lower edge); dorsal coloration in adults green with pale blue trim on anterior edges of dorsal scales, and pale blue postocular stripe with green along the keels in center of stripe; and iris pale green, pale gray, or pale tan.

Figure 2. Photographs in life of the adult male holotype of *Bothriechis guifarroi* (UTA R-60303), with lateral and dorsal views of the head. Photographs by JHT.

Diagnosis. *Bothriechis guifarroi* can be distinguished from the other members of the genus *Bothriechis* as follows (*B. guifarroi* features indicated first, those for species compared next): *B. aurifer* (distributed at moderate and intermediate elevations from extreme east-central Chiapas, Mexico, to east-central Guatemala) — adult color pattern (green vs. black-bordered yellow blotches on green background and prominent black postocular stripe) and juvenile color pattern (green with pale blue blotches or brown with pale paraventral stripe and dark dorsal blotches vs. pale lime green with black-bordered yellow blotches); *B. bicolor* (occurring marginally at low upward to intermediate elevations from southeastern Chiapas, Mexico, to south-central Guatemala) — number of dorsal scales at midbody (19 vs. 21) and condition of prelacunal and second supralabial scales (fused vs. separate); *B. lateralis* (moderate to marginally high elevations from northwestern Costa Rica to western Panama) — number of dorsal scale rows at midbody (19 vs. modal number of 23), adult color pattern (green vs. green with pale paravertebral bars and paraventral stripe), and juvenile color pattern (bi-morph pattern of green with blue dorsal blotching or brown with pale paraventral stripe and short unicolor dark blotches vs. uni-morph pattern of brown ground color with pale paraventral stripe and short bicolor dark and pale blotches); *B. marchi* (found marginally at low elevations up to intermediate elevations in northwestern Honduras and adjacent Guatemala) — condition of prelacunal and second supralabial scales (fused vs. separate), number of subcaudals in females (60–63 vs. 46–57); *B. nigroviridis* (moderate to intermediate elevations from north-central Costa Rica to west-central Panama) — adult color pattern (patternless green vs. green with very heavy black mottling), juvenile color pattern (green with pale blue blotches or brown with pale paraventral stripe and a series of short darker dorsal blotches vs. green with heavy black mottling), iris color (pale green, pale gray, or pale tan vs. almost black), numbers of ventral scales in both sexes (162–166 and 158–166 vs. 143–158 and 134–158), numbers of subcaudal in both sexes (60–68 and 60–63 vs. 49–56 and 44–58), and condition of prelacunal and second supralabial scales (fused vs. separate); *B. rowleyi* (moderate to intermediate elevations from extreme southeastern Oaxaca to northwestern Chiapas, Mexico) — condition of prelacunal and second supralabial scales (fused vs. almost always separate), iris color (pale green, pale gray, or pale tan vs. yellow), and juvenile color pattern (green with pale blue blotches or brown with pale paraventral stripe and a series of short darker dorsal blotches vs. pale yellowish green with brown or purple dorsal blotches); *B. schlegelii* (low to intermediate elevations from northwestern Chiapas, Mexico, southward through Central America and into northwestern South America as far as extreme western Venezuela and extreme northern Peru) — lack of superciliary scales in the former and their presence in the latter, number of supralabials (10–12, usually 10 vs. 7–10, usually 8), number of midbody dorsal scale rows (19 vs. 21–25, usually 23), and adult color pattern (green vs. extremely variable color and pattern involving ground color of yellow, pink, brown, gray, or green and dorsal blotching of a sizable array of colors, but sometimes absent; contrasting postocular stripe absent vs. present); *B. supraciliaris* (moderate to intermediate elevations from southwestern Costa Rica to west-central Panama) — lack of superciliary scales in the former and their presence in the latter, number of midbody dorsal scale rows (19 vs. 21–23, usually 23), number of ventral scales in both sexes (162–166 and 158–166 vs. 145–150 and 141–148), number of subcaudal scales in both sexes (60–68 and 60–63 vs. 48–54 and 45–52), and adult color pattern (green vs. extremely variable color and pattern involving ground color of shades of green, brown, or maroon and dorsal blotching of an array of colors contrasting with that of the ground color; contrasting postocular stripe absent vs. present); *B. thalassinus* (moderate to intermediate elevations from extreme eastern Guatemala and extreme northwestern El Salvador to western Honduras) — number of midbody dorsal scale rows (19 vs. 21–23, usually 21), and condition of prelacunal and second supralabial scales (fused vs. separate).

Description of holotype. An adult male (Figs 2, 3) with hemipenes partially everted, left removed; rostral broader than high (4.38 × 3.26 mm); 2 internasals an-

Figure 3. Dorsal, lateral, and ventral aspects of the head of the holotype of *Bothriechis guifarroi* (UTA R-60303). Photographs by RCJ.

teriorly; 2/2 canthals; 4 posterior intercanthals; supraoculars slightly more than two times as long as broad; 5 intersupraoculars; many scales on head of large size, including large, flat frontal and parietal scales; interrictals 25; single loreal, longer than high, bounded by upper two preoculars, canthal above, prelacunal and prefoveals below, and nasal; prefoveals 3/3, subfoveals 1/1; prelacunal and second supralabial fused; preoculars 3/3, upper largest, middle large and in contact with supralacunal; suboculars 2/2; postoculars 2/2; supralabials 10/10; mental broader than long (4.39 x 3.29 mm); infralabials 11/11; chin shields contacting first four pairs of infralabials; gulars between chin shields and first preventral 6/4; dorsal scale rows 19-19-15; preventrals 2; ventrals 161; cloacal scute undivided; 65 undivided subcaudals; tail spine short and blunt.

Measurement of holotype. Total length 734 mm; tail length 136 mm, comprising 18.5% of total length; head 29.8 mm from front face of rostral to posterior end of mandible; head 19 mm at broadest point; neck 7 mm directly behind jaws.

Hemipenis description of holotype. The partially everted left hemipenis of the holotype is described. Hemipenis at least 20 mm in total length and 13 mm in maximum width at level of crotch; on sulcate side base with several rows of small spines (< 0.5 mm) followed by rows of larger spines and hooks extending for 5 mm, largest protruding ca. 3.5 mm; asulcate side with minutely spined base up to 7 mm before level of bilobation; numerous small mesial spines (< 0.5 mm) arranged in rows present for 4 mm to the calyces, with peripheral section of each lobe containing nine spines and hooks (≥ 2 mm), five of which border lower rim of calyces; calyces follow spines and hooks distally; calyces scalloped, at least 10 rows at least 7 mm to apex of hemipenis on asulcate side; sulcus spermaticus deep and bifurcating ca. 4 mm before site of bilobation and extending upwards through spines and calyces likely to tip of each lobe; sulcus spermaticus bordered by two, occasionally three, columns of minute scales to the beginning of calyces, which form the border likely to the apex of the lobes. Although the majority of the hemipenial characters of this specimen are reported, the lack of a fully everted hemipenis leaves information on the total length and the nature of the calyces incomplete.

Coloration of holotype in life. Middorsal scales of the holotype Yellowish Spectrum Green (Color 128), fading to Light Grass Green (Color 109) laterally and becoming Chartreuse (Color 89) ventrolaterally, with Medium Greenish Yellow (Color 88) ventral scales; dorsal body scales edged anteriorly in Light Caribbean Blue (Color 163), with up to approximately one-fourth of the anterior end of some scales edged in blue; skin concealed between dorsal scales Spectrum Violet (Color 186); postoccipital stripe Light Caribbean Blue (Color 163), with keel and adjacent portion of three scales that lie within the postoccipital stripe Light Emerald Green (Color 142); terminal portion of tail Plumbeous (Color 295); iris Pale Bluish Gray (Color 287) with fine black reticulations most heavily concentrated around the pupil.

Color pattern of holotype in preservative. Scales on dorsal surfaces of the head and body blue-green, becoming more green laterally and yellow-green to yellow ventrally. Tail is mostly green with some grayish blue-green at the dorsal base. Pupil is cloudy and pale, surrounded by lime green iris heavily speckled with black.

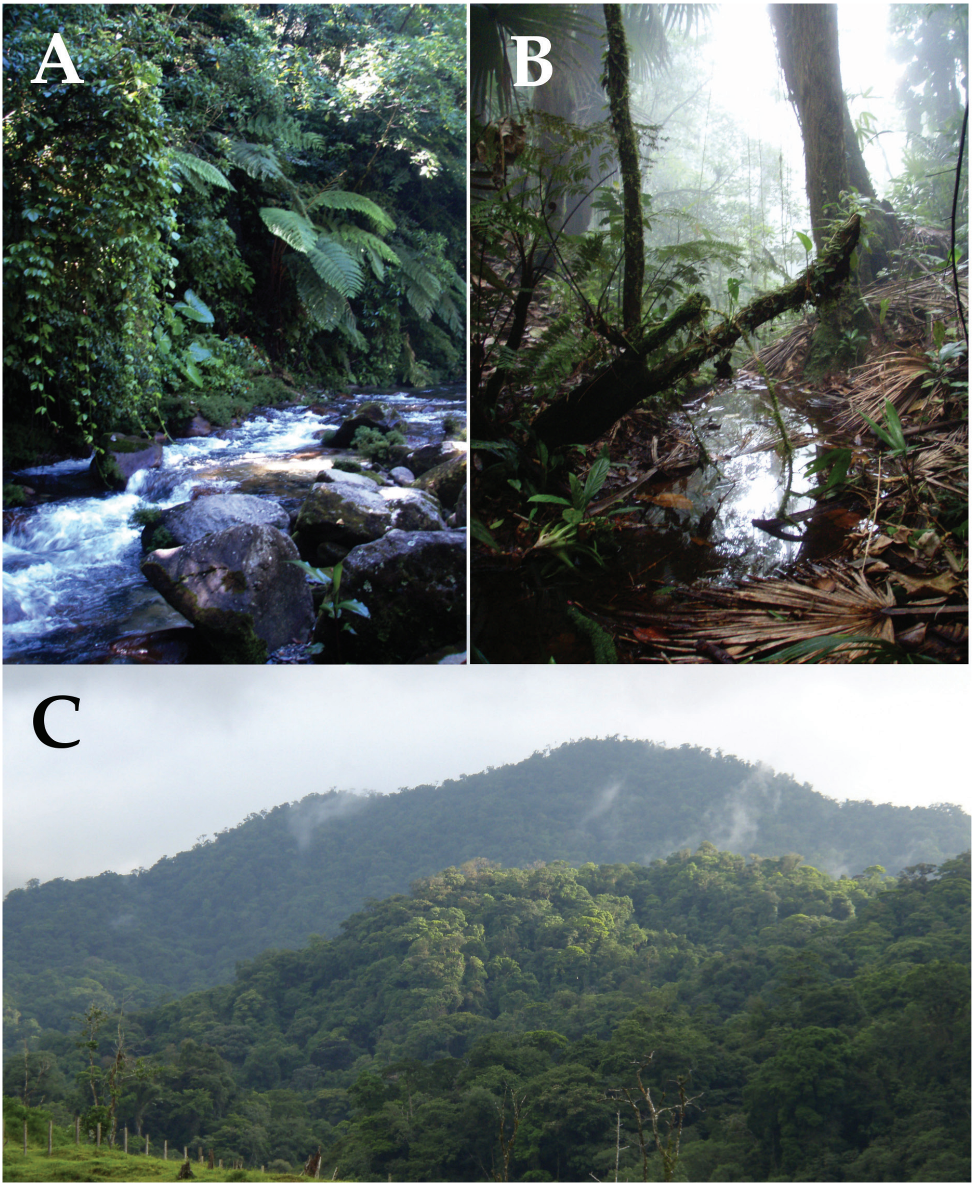

Figure 4. Habitat in the vicinity of the type locality of *Bothriechis guifarroi,* La Liberación, Refugio de Vida Silvestre Texíguat, Honduras; **A** riparian vegetation along the Río Jilamito, 1,015 m elevation **B** small seepage pond near the top of Cerro El Chino, 1,380 m elevation **C** premontane rainforest with the clearing around La Liberación (1,030 m elevation) visible in the foreground. Photographs by JHT.

Variation in paratypes. We discuss scutellational variation in the three adult male paratypes first, the three adult females next, and finally the two unsexed neonates. Scutellation varies as follows (range followed by mean): ventrals (162–166 [164.3], 158–166 [161.7], 162 and 166); subcaudals (60–68 [64.0], 60–63 [61.0], 62 and 68); ventrals +

subcaudals (222–233 [228.3], 221–226 [222.7], 228 and 230); cloacal scute entire in all specimens; dorsal scale row formula 19-19-15, with the reduction to 15 rows occurring at ventrals 114–162; supralabials (10–11 [10.2], 10—11 [10.2], 10–10 and 12–11); infralabials (10–12 [11.0], 10–13 [11.7], 11–11 and 12–11); preoculars 2–2 in all specimens, except 3-3 in CM 156870; postoculars 2–2 in all specimens, except 3–2 in MVZ 269305 and 4–4 in CM 156870; suboculars 2–3 [2.5], 2–3 [2.7], 2–2 and 3–4; relative tail length (0.184–0.223 [0.199], 0. 167–0.182 [0.176], 0.179 and 0.194).

Two juvenile color patterns are present in this species (Fig. 5), one we refer to as a "green phase", the other as a "brown phase." Both juvenile phases have distinctively colored tail-tips, presumably used in caudal luring, and well-differentiated postocular stripes. The green phase (USNM 579874) has a Chartreuse (Color 89) dorsal ground color with Light Turquoise Green (Color 146) edging on the dorsal scales as well as on a series of irregular middorsal blotches, a Pale Green (Color 99) venter, and a Chartreuse (Color 89) head with a Jet Black (Color 300) postocular stripe, bordered above and below by Light Turquoise Green (Color 146); tip of tail Cobalt Blue (Color 180); the iris is Pale Neutral Gray (Color 296) with fine darker reticulations. The brown phase (USNM 579873) has a Robin Rufous (Color 29) ground color middorsally and anteriorly, becoming Salmon Color (Color 58) laterally and posteriorly, with a series of irregular Ferrunginous (Color 35) middorsal blotches, a Pale Buff (Color 1) ventral surface of head and venter becoming gradually darker (Pale Pinkish Buff [Color 3]) posteriorly, a Dark Salmon Color (Color 59) head with a Chestnut (Color 30) postocular stripe, bordered above and below by Light Buff (Color 2); tip of tail Sepia (Color 286); a Pale Buff (Color 1) paraventral stripe is present on the lower half of the first dorsal scale row and the lateral edge of the ventrals; dark speckling along lateral edge of the ventrals; the iris is Chamois (Color 84) with fine darker reticulations.

The type series of *B. guifarroi* demonstrates considerable variation in the condition and shape of the scales on the dorsal surface of the head (Fig. 6), a characteristic often considered diagnostic among *Bothriechis* (Campbell and Smith 2000, McCranie 2011a). Two adult females (USNM 579875 [Fig. 6C] and USNM 579877 [Fig. 6A]), one adult male (MVZ 269305 [Fig. 6B]), and one neonate (USNM 579873 [Fig. 6F]) all have multiple enlarged, unkeeled, plate-like scales present anterior to the posteriormost edge of the orbits; one adult female ([Fig. 6D]), one adult male ([Fig. 6E]), and one neonate (Fig. 6G) all have smaller keeled scales present anterior to the posteriormost edge of the orbits. As a result of demonstrating essentially the full range of dorsal head scale conditions in the type series, we do not consider this characteristic to be of value in diagnosing *B. guifarroi* from other congeners.

Etymology. The specific name *guifarroi* is a patronym used to honor our colleague and friend, Honduran environmental leader Mario Guifarro of Olancho. Don Mario fearlessly led grassroots efforts to stop illegal logging in the indigenous Tawahka territory of eastern Honduras, despite repeated assassination attempts and threats on his own life and those of his compatriots. Don Mario was murdered on 15 September 2007, ironically Honduras' Independence Day, while leading a mission to demarcate the boundaries of the Tawahka-Asangni Biosphere and stave off further illegal defor-

Figure 5. Paratypes of *Bothriechis guifarroi* in life; **A** USNM 579874, green-phase juvenile **B** USNM 579873, brown-phase juvenile **C** close-up of head of USNM 579874 **D** close-up of head of USNM 579873 **E** USNM 579875, female paratype photographed in situ at Cerro El Chino, 1,420 m elevation. Photographs by JHT.

estation. On 21 July 2008, the only witness to Mario's assassination, his son Shamir Guifarro Ramírez, was also murdered, along with Mario's father-in-law, Henry Arturo Chacón, and mother-in-law, Nelda Ochoa, after they were followed out of the city of Juticalpa by unknown assailants.

Distribution. Populations genetically confirmed to represent *Bothriechis guifarroi* are found between 1,015–1,450 m elevation in the western portion of the Cordillera Nombre de Dios, Department of Atlántida, Honduras, within the boundaries of Refugio de Vida Silvestre Texíguat (Fig. 7). These localities lie within the Premontane Wet Forest and peripherally in the Lower Montane Wet Forest formations of Holdridge (1967; as applied by McCranie and Wilson 2002).

Natural history. The holotype was found coiled at 2130h approximately 2.5 m above the ground among old leaf sheaths in the crown of a medium-sized understory

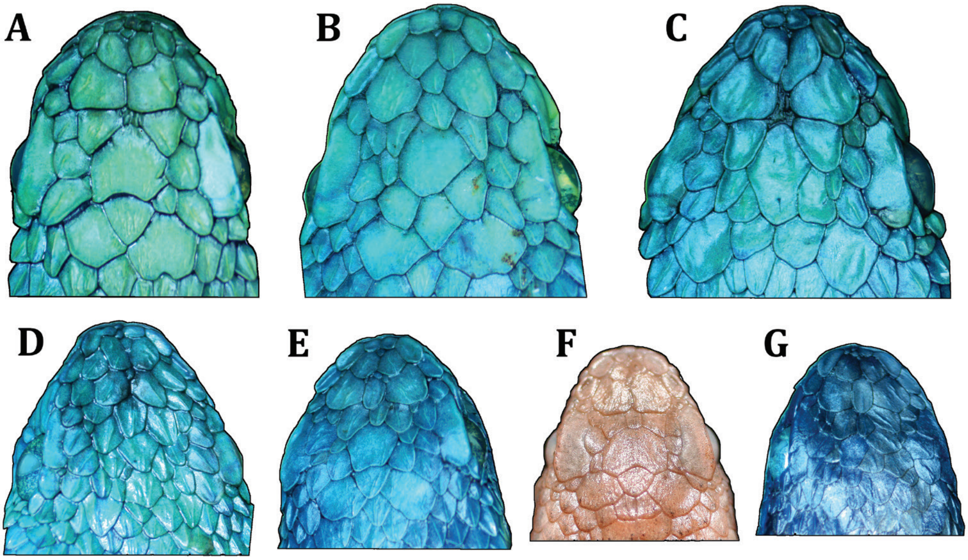

Figure 6. Variation in dorsal head scales among paratypes of *Bothriechis guifarroi*; **A** USNM 579877, adult female **B** MVZ 269305, adult male **C** USNM 579876, adult female **D** USNM 579875, adult female **E** USNM 579878, adult male **F** USNM 579873, neonate **G** USNM 579874, neonate. Photographs by JHT.

palm in gallery forest alongside the Río Jilamito (Fig. 4A). Anurans of the genera *Duellmanohyla* and *Ptychohyla* were abundant in the immediate vicinity of the holotype. Two adult males (CM 156870 and MVZ 269305) were collected along a ridge on the north side of La Liberacíon on the night of 28 July 2010. CM 156870 was active on a small tree from 0.5–1.5 m above the ground; the second snake (MVZ 269305) was sitting coiled on the ground at the edge of the trail, and attempted to escape by crawling across the path when we approached. In the immediate vicinity of these snakes were numerous *Craugastor rostralis* active on the ground and *Bolitoglossa* sp. active on low vegetation. Two neonates were collected on the same night (2100–2200 h) on 18 June 2010, and an adult female (USNM 579875) was collected the next night, in an area of elfin forest at 1,380 m on the ridge called Cerro El Chino (Fig. 4B) above the remote ranch locality La Liberación (at 1,030 m). The brown-phase neonate (USNM 579873) was found atop a large, similarly-colored dead palm frond, while the green-phase neonate (USNM 579874) was sitting in essentially the same ambush position as USNM 579873, but on top of a living green frond. Amphibian species collected in the immediate vicinity of *B. guifarroi* include *Bolitoglossa* sp., *Nototriton* sp., *Craugastor rostralis*, *Plectrohyla chrysopleura*, and *Ptychohyla spinipollex*. Twelve *Bolitoglossa* sp. were encountered the same night as the two neonates, all while active on or around dead and living palm fronds in the immediate vicinity of the neonates.

Remarks. Townsend et al. (2012: 107) included a photograph of the holotype of *Bothriechis guifarroi* as "*Bothriechis marchi.*" *Bothriechis guifarroi* is typically distinguished from *B. marchi* by having the prelacunal scale fused to the second supralabial;

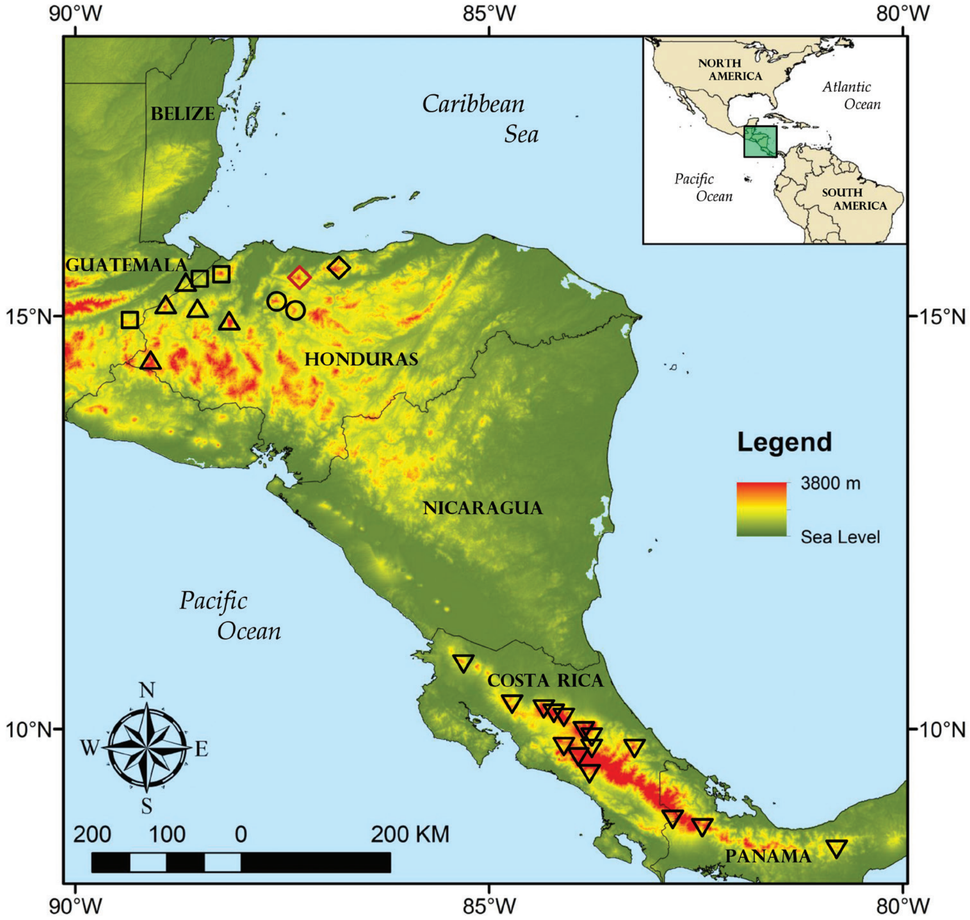

Figure 7. Geographic distribution of selected *Bothriechis* species and populations discussed in the text; localities are based on data published herein and those of Campbell and Lamar (2004), McCranie (2011a), and Savage (2002); red diamond = type locality of *B. guifarroi* in Refugio de Vida Silvestre Texíguat; black diamond = referred population of *B. guifarroi* from Parque Nacional Pico Bonito; circles = *Bothriechis sp. inquirenda* populations for the Sierra de Sulaco; squares = *B. marchi*; triangles = *B. thalassinus*, inverted triangles = *B. lateralis*.

however, one male paratype of *B. guifarroi* (CM 156870) has the right prelacunal separated from the second supralabial (they are fused on the left side). Also, one specimen of *B. marchi* (MCZ R-33335) from the "mountains west of San Pedro Sula" also has fused prelacunals and second supralabials on both sides, and another specimen (MCZ R-32030) from "La Cumbre" has the left prelacunal separated from the second supralabial (with them fused on the right side).

We tentatively refer four additional specimens to *Bothriechis guifarroi*: two from a locality on the leeward side of Refugio de Vida Silvestre Texíguat (USNM 337488–89, from 2.5 airline km NNE of La Fortuna, Dept. Yoro, 1,550 m elevation), one from the central portion of the Cordillera Nombre de Dios (USNM 319942, from Quebrada de Oro in Parque Nacional Pico Bonito, Dept. Atlántida, 1,090 m elevation), and one from "Tela" (AMNH 46949). All four specimens also have fused prelacunals and second supralabials on both sides. USNM 319942 was collected as a juvenile and raised in captivity (Wilson and McCranie 1992; McCranie 2011a), and exhibited a similar juvenile color pattern as USNM 579873 before undergoing an ontogenetic shift in coloration to the bright green pattern exhibited by the type series of *B. guifarroi*. Campbell and Lamar (2004: plates 385–386) also provided illustrations of juvenile *B. marchi s.l.* that demonstrated two color morphs similar to those exhibited by *B. guifarroi*; however these two individuals were captive born in the Houston Zoo and known only from "Honduras."

AMNH 46949 was collected sometime during or before 1932 by Douglas March of the Lancetilla Serpentarium, just outside of the seaside city of Tela. While the "Tela" locality is considered erroneous (Wilson and McCranie 1992), it is possible that AMNH 46949 was obtained from somewhere in the nearby western portion of the Cordillera Nombre de Dios. Given that at least some highland taxa found at both RVS Texíguat and Parque Nacional Pico Bonito are endemic sister species (e.g., *Oedipina gephyra* and *O. petiola*; McCranie and Townsend 2011), we refer USNM 319942 to *B. guifarroi* with the understanding that phylogenetic evaluation of the Pico Bonito population might eventually show those animals to represent a distinct taxon.

Discussion

Conservation status of *Bothriechis guifarroi*. With the description of *Bothriechis guifarroi*, there are now at least three species of palm pitvipers endemic to the Chortís Highlands, including *B. marchi* and *B. thalassinus*, with the potential for additional undescribed taxonomic diversity pending phylogenetic evaluation of allopatric populations in central Honduras. Based on the IUCN Red List criteria (2012), *B. guifarroi* should be classified as Critically Endangered (B1ab[iii]+2ab[iii]) due to its limited known area of occurrence and the potential for anthropogenic damage to its habitat. According to the algorithm developed by Wilson and McCranie (2004a), we calculated the Environmental Vulnerability Score (EVS) for *B. guifarroi* as 5+8+5=18, allocating it to the category of a high vulnerability species. Given this conservation status, *B. guifarroi* becomes the 48th member of the critically endangered endemic component of the Honduran herpetofauna (Wilson et al. 2012), and the tenth snake species and the first viperid species so designated. This species also warrants immediate consideration for protection under CITES, given its potential for exploitation in the pet trade.

The vicinity of the type locality of *B. guifarroi* is part of the relatively large and intact premontane rainforests and cloud forests of Refugio de Vida Silvestre Texíguat, one of the most important areas of herpetofaunal endemism in Mesoamerica (Townsend et al. 2012). While deforestation in the leeward portion of Refugio de Vida Silvestre Texíguat has been documented since at least the early 1990's (see summary in Townsend et al. 2010), Townsend et al. (2012) reported that the windward portion of the reserve contained a large intact expanse of virtually undisturbed forest. In late 2012, a plot was cleared in the upper Río Jilamito watershed to the south of La Liberación, marking the first time farmers from adjacent Yoro had crossed into the Río Jilamito watershed and illegally cleared land (L. Herrera-B., pers. comm.). This is an ominous development, particularly in light of the recent drastic reduction in financial support for conservation efforts in Refugio de Vida Silvestre Texíguat, which had funded the training and employment of a team of local park guards during 2010–2012.

Herpetofaunal Endemism in the Chortís Highlands. Whereas Nuclear Central America has long been accepted as a region of high biodiversity and endemicity, some observers have further recognized the western and eastern portions of this highland block as distinct biogeographic entities (Johnson 1989; Campbell 1999; Townsend 2006, 2009). Eastern Nuclear Central America has been shown to have a distinctive component of endemic biodiversity, particularly in amphibians and reptiles (Wilson and Johnson 2010); however, molecular characterization of evolutionary diversification patterns in this region has been limited to a few studies of a restricted taxonomic breadth and broader geographic focus (e.g. Castoe et al. 2009). This region is geographically analogous to the Chortís Block, an allochthonous geological formation that currently forms the only modern continental portion of the Caribbean Tectonic Plate and the largest terrestrial segment of the contemporary Central American land bridge (Rogers 2003; Marshall 2007). The Chortís Block has a challengingly complex history, and recently has been the subject of increased focus, and sometimes contentious debate, within the geological research community (James 2007; Mann et al. 2007; Ortega-Gutiérrez et al. 2007; Silva-Romo 2008; Morán-Zenteno et al. 2009).

The majority of the geographical extent of the Chortís Highlands is found within the political boundaries of Honduras, the country with the highest degree of herpetofaunal endemism of any Central American nation (Wilson and Johnson 2010). Townsend and Wilson (2010) reported 91 endemic species (47 amphibians and 44 reptiles) from Honduras. Since that work went to press, an additional ten endemic species have been described from Honduras, including three new plethodontid salamanders (*N. picucha*, Townsend et al. 2011; *N. tomamorum*, Townsend et al. 2010; *Oedipina petiola*, McCranie and Townsend 2011), a new black iguana (*Ctenosaura praeocularis*, Hasbún and Köhler 2009), a new skink (*Marisora roatanae*; Hedges and Conn 2012), two new dwarf geckos (*Sphaerodactylus guanajae* and *S. leonardovaldesi*; McCranie and Hedges 2012), and three new colubrid snakes (*Omoadiphas cannula*, McCranie and Cruz-Díaz 2011; *Tantilla psittaca*, McCranie 2011b; *Tantilla olympia*, Townsend et al. 2013). With these species included, the total stands at 101 species, making *B. guifarroi* the 102nd described herpetofaunal species endemic to Honduras.

***Bothriechis marchi* and the status of populations from Yoro.** We recognize *Bothriechis marchi sensu stricto* as occurring in localities in the Cordillera de Merendón in the Honduran departments of Cortés and Santa Bárbara along the border with Guatemala, as well as for at least one isolated locality in eastern Guatemala (Fig. 7). This Guatemalan locality, Cerro del Mono in Departamento de Zacapa, previously was the source of the only sequenced sample assigned to *B. marchi* (UTA R-52959; Castoe and Parkinson 2006). Although the population of *Bothriechis* from Cerro del Mono does not agree morphologically with the typical form of *B. marchi* (E.N. Smith, pers. comm.; see Plates 422–424 in Campbell and Lamar 2004, as *B. thalassinus*), the sequence data attributed to UTA R-52959 are not notably divergent from a sample of typical *B. marchi* (MVZ 263604) collected from the Sierra de Omoa in northern Honduras (Fig. 1).

The type localities of *Bothriechis marchi* and *B. thalassinus* are both in the Sierra de Caral, within approximately 20 km of one another on opposite sides of the Guatemala/Honduras border (Campbell and Lamar, 2004) suggesting that the two species occur either in parapatry or sympatry in the limited forest remaining in that mountain range (the type locality of *B. marchi* is not precise; Wilson and McCranie [1992] restricted it to the forested hills above El Oro, Departamento de Santa Bárbara). Individuals of both taxa in the Sierra de Caral exhibit a primarily green dorsal coloration, with some scattered bluish middorsal blotches (Campbell and Smith, 2000; Campbell and Lamar, 2004: plate 425). While the presence of *B. thalassinus* has not been confirmed by vouchered specimens from the Sierra de Omoa, the proximity of the Sierra de Caral and the Sierra de Omoa in northwestern Honduras and the similarity in coloration exhibited between these nominal taxa in that vicinity suggest the possibility that *B. thalassinus* may have gone unnoticed in the Sierra de Omoa. We have evaluated photographs of over a dozen individuals of green *Bothriechis* from the Sierra de Omoa encountered as part of an expedition-tourism operation in that area over the past five years, and have noted considerable variation in head scalation in the photographs. Unfortunately, none of the photographed individuals were collected nor were genetic samples taken to allow for more detailed evaluation of the *Bothriechis* of the Sierra de Omoa. In addition to the nominal form of *B. marchi*, it is likely that more than one species of *Bothriechis* occurs in sympatry or parapatry in the Sierra de Omoa, possibly including *B. thalassinus* and/or an unidentified sister taxon of *B. guifarroi*. Focused sampling and phylogenetic analysis of *Bothriechis* from the Sierra de Omoa is needed to better characterize the taxonomic diversity present in that mountain range.

Paraphyly in *Bothriechis marchi sensu lato* in terms of populations from the Cordillera de Merendón and the Cordillera Nombre de Dios, the latter now known to represent *B. guifarroi*, calls into question the taxonomic status of populations from isolated localities in the Sierra de Sulaco in Departmento de Yoro (Fig. 7). These populations are represented in collections by one specimen from Cerro de Pajarillos (USNM 561085), three specimens from the Montaña de Mataderos (FMNH 21777, MCZ R-38785–86), 14 specimens from "Portillo Grande" near Montaña Macuzal (FMNH 34732–35, 35895, 35999–601, 37217, 38542, 41621; MCZ R-38789–91), and two specimens from "Subirana Valley" (FMNH 21892, MCZ R-38788). Four of these specimens examined by us for this paper (MCZ R-38785–86, 38790–91) differ from *B. guifarroi* in having 21–19–15 dorsal scale rows (versus 19–19–15) and having varying conditions of fusion of the prelacunal and second supralabial (fused on both sides in MCZ R-38790, separate on both sides in MCZ R-38786, and fused on one side and separate on the other in MCZ R-38785 an R-38791). Given the considerable phylogenetic diversification presented by analysis of *B. guifarroi* and *B. marchi s. s.*, we cannot justify assignment of the Yoro populations to either taxon in the absence of molecular characterization of those populations. Therefore, we tentatively refer to the Yoro populations as *Bothriechis species inquirenda* pending collection of fresh material and phylogenetic characterization. Efforts to secure this material are currently underway.

Evolutionary and biogeographic implications. The phylogenetic position of *B. guifarroi* has significant implications for our understanding of the biogeography and evolution of palm-pitvipers. Crother et al. (1992) first presented a phylogenetic hypothesis for the genus *Bothriechis* supporting an eyelash pitviper clade (*B. schlegelii* and *B. supraciliaris*) that is sister to a clade containing the remaining species of *Bothriechis*, all of which have highland-associated distributions (typically <1,000 m elevation; Fig. 7). Castoe et al. (2009) and Daza et al. (2010) provided the most recent phylogenetic estimates for nine species of *Bothriechis*, as part of a comparative phylogeographic study of three co-distributed, predominantly Mesoamerican genera of pitvipers. Their phylogenetic hypotheses supported the contention of Castoe and Parkinson (2006) that *Bothriechis lateralis* is the sister group to the Nuclear Central American highland species of the genus (*B. aurifer*, *B. bicolor*, *B. marchi*, *B. rowleyi*, and *B. thalassinus*) and rejected the Crother et al. (1992) and Taggart et al. (2001) estimates that *B. lateralis* is the sister clade to *B. bicolor*. Our phylogenetic hypothesis also supports a Nuclear Central American clade, with *B. marchi* and *B. thalassinus* in the Chortís Highlands comprising a group that is sister to *B. aurifer*, *B. bicolor*, and *B. rowleyi* from the Nuclear Central American highlands to the west.

The Cordillera Nombre de Dios, which includes Refugio de Vida Silvestre Texíguat and Parque Nacional Pico Bonito, is home to a distinct endemic biota that, in many cases, bears little resemblance to the endemic communities found in other nearby cloud forests (Wilson and McCranie 2004b). While published phylogenetic datasets of taxa endemic to the Cordillera Nombre de Dios are limited, evidence is beginning to accumulate that this region, and Refugio de Vida Silvestre Texíguat in particular, represents a paleo-refugium where relict lineages have accumulated and persisted while disappearing elsewhere in northern Central America. *Bothriechis guifarroi* provides the best evidence to date in support of the paleo-refugium hypothesis, given its phylogenetic relationship with *B. lateralis* and *B. nigroviridis* from southern Central America and lack of close evolutionary relationships with other taxa from Nuclear Central America. Another recently described species from Refugio de Vida Silvestre Texíguat, the moss salamander *Nototriton tomamorum*, is morphologically and phylogenetically more closely associated with the Costa Rican taxon *N. richardi* than it is with members of the northern *N. barbouri* group, which includes an undescribed taxon that occurs in sympatry with *N. tomamorum* (Townsend et al. 2010). *Isthmohyla insolita*, another Texíguat endemic, is one of only two *Isthmohyla* in northern Central America (the other being *I. melacaena* from the Sierra de Omoa; neither species has been subjected to phylogenetic analysis), with the remaining 13 species restricted to highlands of Costa Rica and Panama (Köhler 2011).

Castoe et al. (2009) estimated that Lower Central American and Nuclear Central American *Bothriechis* diverged at the Nicaraguan Depression between 5.73–9.87

mybp, based on the hypothesis that *B. lateralis* represented the sister lineage to the Nuclear Central American clade. Given the discovery of *B. guifarroi*, the timing of divergence across the Nicaraguan Depression could be much more recent, similar to that seen between *Cerrophidion sasai* and *C. wilsoni* (3.06–6.03 mybp; Castoe et al. 2009). Alternatively, it is possible that a Lower Central American *Bothriechis* (i.e., *B. guifarroi*, *B. lateralis*, *B. nigroviridis*) may have split from Nuclear Central American taxa via the Nicaraguan Depression earlier than suggested by Castoe et al. (2009) and Daza et al. (2010). Following additional sampling and the evaluation of populations presently referred to as *B. sp. inquirenda*, a reevaluation of Mesoamerican pitviper biogeography is warranted, and may shed further light on the complicated biogeographic relationship between northern and southern Central America.

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References

Arévalo ES, Davis SK, Sites JW Jr. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Systematic Biology 43: 387–418. doi: 10.1093/sysbio/43.3.387

Barbour T, Loveridge A (1929) On some Honduranian and Guatemalan snakes with the description of a new arboreal pit viper of the genus *Bothrops*. Bulletin of the Antivenin Institute of America 3: 1–3.

Campbell JA (1982) The biogeography of the cloud forest herpetofauna of Middle America, with special reference to the Sierra de las Minas of Guatemala. PhD thesis, Lawrence, Kansas: University of Kansas.

Campbell JA (1999) Distribution patterns of amphibians in Middle America. In: Duellman WE (Ed) Patterns of Distribution of Amphibians. A Global Perspective. Johns Hopkins University Press, Baltimore, Maryland, 111–210.

Campbell JA, Flores-Villela O (2008) A new long-tailed rattlesnake (Viperidae) from Guerrero, Mexico. Herpetologica 64: 246–257. doi: 10.1655/07-054.1

Campbell JA, Lamar WW (2004) The Venomous Reptiles of the Western Hemisphere. 2 vols. Comstock Publishing Associates, Cornell University Press, Ithaca, New York. xviii + 870 + 28 pp.

Campbell JA, Smith EN (2000) A new species of arboreal pitviper from the Atlantic versant of northern Central America. Revista de Biología Tropical 48: 1001–1013.

Cadle JE (2012) Cryptic species within the *Dendrophidion* complex in Middle America (Serpentes: Colubridae). Bulletin of the Museum of Comparative Zoology 160: 183–240. doi: 10.3099/0027-4100-160.4.183

Cadle JE, Savage JM (2012) Systematics of the *Dendrophidion nuchale* complex (Serpentes: Colubridae) with the description of a new species from Central America. Zootaxa 3513: 1–50.

Castoe TA, Parkinson CL (2006) Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes). Molecular Phylogenetics and Evolution 39: 91–110. doi: 10.1016/j.ympev.2005.12.014

Castoe TA, Sasa MM, Parkinson CL (2005) Modeling nucleotide evolution at a mesoscale: the phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: Crotalinae). Molecular Phylogenetics and Evolution 37: 881–898. doi: 10.1016/j.ympev.2005.05.013

Castoe TA, Daza JM, Smith EN, Sasa MM, Kuch U, Campbell JA, Chippindale PT, Parkinson CL (2009) Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. Journal of Biogeography 36: 88–103. doi: 10.1111/j.1365-2699.2008.01991.x

Crother BI, Campbell JA, Hillis DM (1992) Phylogeny and historical biogeography of the palm-pitvipers, genus *Bothriechis*: Biochemical and morphological evidence. In: Campbell JA, Brodie ED Jr (Eds) Biology of the Pitvipers. Selva, Tyler, Texas, 1–20.

Daza JM, Castoe, TA, Parkinson CL (2010) Using regional comparative phylogeographic data from snake lineages to infer historical processes in Middle America. Ecography 33: 343–354.

Dowling HG, Savage JM (1960) A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. Zoologica 45: 17–30.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. doi: 10.1093/nar/gkh340

Hasbún CR, Köhler G (2009) New species of *Ctenosaura* (Squamata, Iguanidae) from southeastern Honduras. Journal of Herpetology 43: 192–204. doi: 10.1670/08-039R1.1

Hedges SB, Conn CE (2012) A new skink fauna from Caribbean islands (Squamata, Mabuyidae, Mabuyinae). Zootaxa 3288: 1–244.

Holdridge LR (1967) Life Zone Ecology. Second Edition. San José, Costa Rica. 206 pp.

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second Edition. IUCN, Gland, Switzerland and Cambridge, UK, iv + 32pp.

Jadin RC, Smith EN (2010) The hemipenis of *Trimorphodon quadruplex*. Herpetological Review 41: 157–159.

Jadin RC, Smith EN, Campbell JA (2011) Unravelling a tangle of Mexican serpents: a systematic revision of highland pitvipers. Zoological Journal of the Linnean Society, 163: 943–958. doi: 10.1111/j.1096-3642.2011.00748.x

Jadin RC, Townsend JH, Castoe TA, Campbell JA (2012) Cryptic diversity in disjunct populations of Middle American Montane Pitvipers: a systematic reassessment of *Cerrophidion godmani*. Zoologica Scripta 41: 455–470. doi: 10.1111/j.1463-6409.2012.00547.x

James KH (2007) Structural geology: from local elements to regional synthesis. In: Bundschuh J, Alvarado GE (Eds) Central America: Geology, Resources, and Hazards. Taylor and Francis, Oxford, United Kingdom, 277–321.

Johnson JD (1989) A biogeographic analysis of the herpetofauna of northwestern nuclear Central America. Milwaukee Public Museum Contributions in Biology and Geology 76: 1–66.

Keogh JS (1999) Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. Zoological Journal of the Linnean Society 125: 239–278. doi: 10.1111/j.1096-3642.1999.tb00592.x

Knight A, Mindell DP (1993) Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's viper. Systematic Biology 42: 18–31. doi: 10.1093/sysbio/42.1.18

Köhler G (2011) Amphibians of Central America. Herpeton Verlag, Offenbach, Germany, 379 pp.

Köhler G (2012) Color Catalogue for Field Biologists. Herpeton Verlag, Offenbach, Germany, 49 pp.

Mann P, Rogers RD, Gahagan L (2007) Overview of plate tectonic history and its unresolved tectonic problems. In: Bundschuh J, Alvarado GE (Eds) Central America: Geology, Resources, and Hazards. Taylor and Francis, Oxford, United Kingdom, 206–241.

Marshall JS (2007) The geomorphology and physiographic provinces of Central America. In: Bundschuh J, Alvarado GE (Eds) Central America: Geology, Resources, and Hazards. Taylor and Francis, Oxford, United Kingdom, 75–122. doi: 10.1201/9780203947043.pt2

McCranie JR (2011a) The Snakes of Honduras: Systematics, Distribution, and Conservation. Society for the Study of Amphibians and Reptiles 26: i–x, 1–714.

McCranie JR (2011b) A new species of *Tantilla* of the *taeniata* species group (Reptilia, Squamata, Colubridae, Colubrinae) from northeastern Honduras. Zootaxa 3037: 37–44.

McCranie JR, Castañeda FE (2005) The herpetofauna of Parque Nacional Pico Bonito, Honduras. Phyllomedusa 4: 3–16.

McCranie JR, Cruz Díaz G (2010) A third new species of snake of the genus *Omoadiphas* (Reptilia, Squamata, Colubridae, Dipsadinae) from Honduras. Zootaxa 2690: 53–58.

McCranie JR, Hedges SB (2012) Two new species of geckos from Honduras and resurrection of *Sphaerodactylus continentalis* Werner from the synonymy of *Sphaerodactylus millepunctatus* Hallowell (Reptilia, Squamata, Gekkonoidea, Sphaerodactylidae). Zootaxa 3492: 65–76.

McCranie JR, Townsend JH (2011) Description of a new species of worm salamander (Caudata, Plethodontidae, *Oedipina*) in the subgenus *Oedopinola* from the central portion of the Cordillera Nombre de Dios, Honduras. Zootaxa 2990: 59–68.

McCranie JR, Wilson LD (2002) The Amphibians of Honduras. Society for the Study of Amphibians and Reptiles. Contributions in Herpetology 19. Ithaca, New York. i–x, 1–625.

Meyer JR (1969) A biogeographic study of the amphibians and reptiles of Honduras. PhD Thesis, University of Southern California, Los Angeles.

Morán-Zenteno DJ, Keppie DJ, Marinty B, González-Torres E (2009) Reassessment of the Paleogene position of the Chortís Block relative to southern Mexico: hierarchical ranking of data and features. Revista Mexicana de Ciencias Geológicas 26: 177–188.

Myers CW, Cadle JE (2003) On the snake hemipenis, with notes on *Psomophis* and techniques of eversion: a response to Dowling. Herpetological Review 34: 295–302.

Nylander JAA (2004) MrModeltest v2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ortega-Gutiérrez F, Solari LA, Ortega-Obregón C, Elías-Herrera M, Martens U, Morán-Icál, S, Chiquín M, Keppie JD, Torres de León R, Schaaf P (2007) The Maya-Chortís boundary: a tectonostratigraphic approach. International Geological Review 49: 996–1024. doi: 10.2747/0020-6814.49.11.996

Parkinson CL, Moody SM, Ahlquist JE (1997) Phylogenetic relationships of the "*Agkistrodon* complex" based on mitochondrial DNA sequence data. In: Thorpe RS, Wüster W, Malhotra A (Eds) Venomous Snakes; Ecology, Evolution and Snakebite. Clarendon Press, Oxford, England, 63–78.

Parkinson CL, Campbell JA, Chippindale P (2002) Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. In: Scuett GW, Höggren M, Douglas ME, Greene HW (Eds) Biology of the Vipers. Eagle Mountain Publishing, Eagle Mountain, Utah, 93–110.

Rambaut A, Drummond AJ (2009) Tracer v1.5. Available from http://tree.bio.ed.ac.uk/software/tracer/

Rogers RD (2003) Jurassic-Recent Tectonic and Stratigraphic History of the Chortís Block of Honduras and Nicaragua (Northern Central America). PhD Thesis, University of Texas at Austin.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi: 10.1093/bioinformatics/btg180

Rovito SM, Papenfuss TJ, Vásquez-Almazán C (2012) A new species of *Sibon* (Squamata: Colubridae) from the mountains of eastern Guatemala. Zootaxa 3266: 62–68.

Sabaj-Pérez MH (Ed) (2012) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 3.0 (23 February 2012). American Society of Ichthyologists and Herpetologists, Washington, D.C. Available from http://www.asih.org/

Savage JM (2002) The Amphibians and Reptiles of Costa Rica. A Herpetofauna between Two Continents, between Two Seas. Cornell University Press, Ithaca, New York. 934 pp.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90. doi: 10.1139/z91-013

Silva-Romo G (2008) Guayape-Papalutla fault system: A continuous Cretaceous structure from southern México to the Chortís block? Tectonic implications. Geology 36: 75–78. doi: 10.1130/G24032A.1

Smith EN, Ferrari-Castro JA (2008) A new species of jumping pitviper of the genus *Atropoides* (Serpentes: Viperidae: Crotalinae) from the Sierra de Botaderos and the Sierra La Muralla, Honduras. Zootaxa 1948: 57–68.

Solórzano A (2004) Serpientes de Costa Rica, Snakes of Costa Rica. Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica. 791 pp.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. doi: 10.1093/bioinformatics/btl446

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web-servers. Systematic Biology 75: 758–771. doi: 10.1080/10635150802429642

Swofford DL (2002) PAUP*: Phylogenetic analyses using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Taggart TW, Crother BI, White ME (2001) Palm-pit-viper (*Bothriechis*) phylogeny, mtDNA, and consilience. Cladistics 17: 355–370. doi: 10.1111/j.1096-0031.2001.tb00130.x

Townsend JH (2006) Inventory and Conservation Assessment of the Herpetofauna of the Sierra de Omoa, Honduras, with a Review of the *Geophis* (Squamata: Colubridae) of Eastern Nuclear Central America. Master's Thesis, University of Florida, Gainesville, Florida.

Townsend JH (2009) Morphological variation in *Geophis nephodrymus* (Squamata: Colubridae), with comments on the conservation of *Geophis* in eastern Nuclear Central America. Herpetologica 65: 292–302. doi: 10.1655/07-039R2.1

Townsend JH (2011) Integrative Taxonomy Reveals the Chortís Block of Central America as an Underestimated Hotspot of Amphibian Diversity. PhD Thesis, University of Florida, Gainesville, Florida.

Townsend JH, Butler JM, Wilson LD, Austin JD (2010) A distinctive new species of moss salamander (Caudata: Plethodontidae: *Nototriton*) from an imperiled Honduran endemism hotspot. Zootaxa 2434: 1–16.

Townsend JH, Medina-Flores M, Murillo JL, Austin JD (2011) Cryptic diversity in Chortís Higland moss salamanders (Caudata: Plethodontidae: *Nototriton*) revealed using mtDNA barcodes and phylogenetics, with a new species from eastern Honduras. Systematics and Biodiversity 9: 275–287. doi: 10.1080/14772000.2011.611188

Townsend JH, Wilson LD (2008) The Amphibians and Reptiles of Cusuco National Park, Honduras. Los Anfibios y Reptiles del Parque Nacional Cusuco, Honduras. Bibliomania!, Salt Lake City, Utah. i-xiv + 1–312 pp.

Townsend JH, Wilson LD (2010) Conservation of the Honduran herpetofauna: Issues and imperatives. In: Wilson LD, Townsend JH, Johnson JD (Eds) Conservation of Mesoamerican Amphibians and Reptiles. Eagle Mountain Publishing, LC, Eagle Mountain, Utah, 460–487.

Townsend JH, Wilson LD, Medina-Flores M, Aguilar-Urbina E, Atkinson BK, Cerrato-Mendoza CA, Contreras-Castro A, Gray LN, Herrera-B LA, Luque-Montes IR, McKewy-Mejía M, Portillo-Avilez A, Stubbs AL, Austin JD (2012) A premontane hotspot for herpetological endemism on the windward side of Refugio de Vida Silvestre Texíguat, Honduras. Salamandra 48: 92–114.

Townsend JH, Wilson LD, Medina-Flores M, Herrera-BL (2013) A new species of centipede snake in the *Tantilla taeniata* group (Squamata: Colubridae) from premontane rainforest in Refugio de Vida Silvestre Texíguat, Honduras. Journal of Herpetology 47: 191–200. doi: 10.1670/11-172

Williams ST (2007) Safe and legal shipment of tissue samples: does it affect DNA quality? Journal of Molluscan Studies 73: 416–418. doi: 10.1093/mollus/eym039

Wilson LD, Johnson JD (2010) Distributional patterns of the herpetofauna of Mesoamerica, a biodiversity hotspot. In: Wilson LD, Townsend JH, Johnson JD Conservation of Mesoamerican Amphibians and Reptiles. Eagle Mountain Publishing, LC, Eagle Mountain, Utah, 30–235.

Wilson LD, Luque-Montes IR, Alegría AB, Townsend JH (2012) El componente endémico de la herpetofauna hondureña en peligro crítico: priorización y estrategias de conservación. Revista Latinoamericana de Conservación 2(2)-3(1): 47–67.

Wilson LD, McCranie JR (1992) *Bothriechis marchi*. Catalogue of American Amphibians and Reptiles 544.1–544.2.

Wilson LD, McCranie JR (2004a) The conservation status of the herpetofauna of Honduras. Amphibian and Reptile Conservation 3: 6–33.

Wilson LD, McCranie JR (2004b) The herpetofauna of the cloud forests of Honduras. Amphibian and Reptile Conservation 3: 33–48.

Wilson LD, Meyer JR (1985) The Snakes of Honduras. Second edition. Milwaukee Public Museum, Milwaukee, Wisconsin. i–x + 1–150 pp.

Zaher H, Prudente ALC (2003) Hemipenes of *Siphlophis* (Serpentes, Xenodontinae) and techniques of hemipenial preparation in snakes: a response to Dowling. Herpetological Review 34: 302–307.

Appendix

Specimens examined; museum acronyms follow Sabaj-Pérez (2012).

Bothriechis guifarroi (12) – Honduras: ATLÁNTIDA: Quebrada de Oro, USNM 319942; "Tela," AMNH 46949; La Liberación, CM 156870, MVZ 269305, USNM 579873–78, UTA R-60303. YORO: 2.5 airline km NNE of La Fortuna, USNM 337488–89.

Bothriechis marchi (19) – Honduras: CORTÉS: "near Cofradía," MCZ 28014; "La Cumbre," AMNH 46954–57, MCZ 32029–31; Sierra de Omoa, MCZ 33334–36, 33560–64, USNM 83454. SANTA BÁRBARA: "Quimistán," MCZ 27260, 27510; "between Cofradía and Quimistán," MCZ 27567–68, UMMZ 90677 (was MCZ 27569); "Santa Bárbara," MCZ 28014.

Bothriechis thalassinus (3) – Honduras: COPÁN: Quebrada Grande, KU 203094; OCOTEPEQUE: 21.7 km east of Nuevo Ocotepeque, LSUMZ 23821; SANTA BÁRBARA: southeastern slope of Cerro Santa Barbara, LSUMZ 11638.

Bothriechis sp. inquirenda (4) – Honduras: YORO: Montañas de Mataderos, MCZ 38785–86; Portillo Grande, MCZ R-38790–91.