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Article

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A new hidden species of the *Cymothoe caenis*-complex (Lepidoptera: Nymphalidae) from western Africa

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Abstract

Butterflies of the Afrotropical genus *Cymothoe* are characterised by sexual dimorphism. Females of the most common species of the genus, *Cymothoe caenis* Drury, occur in many different forms in the equatorial zone, while only a single form is present west of the Niger Delta in Nigeria, raising the question as to whether the two populations need taxonomic recognition. We present molecular and genitalic evidence that the western populations of *C. caenis* s.l. comprise a distinct species: *Cymothoe druryi* **sp. nov.**

Key words: Biogeography, butterflies, classification, cryptic species, *Cymothoe druryi* **sp. nov.**, Dahomey Gap, DNA barcoding, Limenitidinae, morphology, mtDNA, Niger Delta, Nigeria, sexual dimorphism, taxonomy, west Africa

Introduction

The 'Gliders' (*Cymothoe* Hübner) are a large, entirely Afrotropical butterfly genus of 75 forest species (Ackery *et al.*, 1995; Aurivillius, 1912; Larsen, 2005). Members of the genus are strongly sexually dimorphic (Aurivillius, 1898, 1912) and especially the females can be morphologically variable within species (Aurivillius, 1898, 1912; Schultze, 1917).

The most common and widespread species of the genus is *Cymothoe caenis* Drury (1773), which penetrates open country more effectively than other species and is more at home in secondary habitats than in primary forest (Aurivillius, 1912; Berger, 1981; Larsen, 2005). The female of *C. caenis* is morphologically highly variable within the equatorial zone (Aurivillius, 1912; Birket-Smith, 1960; Larsen, 2005), and this led to the description of almost 20 infraspecific forms (Ackery *et al.*, 1995; Berger, 1981). Conversely, west of the Niger Delta in Nigeria *C. caenis* females are monomorphic, which led Larsen (2005) to hypothesise that this western population needs taxonomic recognition.

Here we present DNA and male genitalic evidence that the western populations of *C. caenis* s.l. constitute a distinct species. The data result from an ongoing genus–wide DNA barcoding project aimed at exploring cryptic species diversity (van Velzen *et al.*, 2007) and at developing DNA barcodes in order to associate immature and adult stages. We assess intra- and interspecific COI DNA sequence divergence of *C. caenis* s.l. and related species, and associate this with their biogeographical distributions, female morphological variation, and male genital differences. A description of *Cymothoe druryi* **sp. nov.** is given here, with prospects for future research on its genetic variation, morphology, biogeography and ecology.

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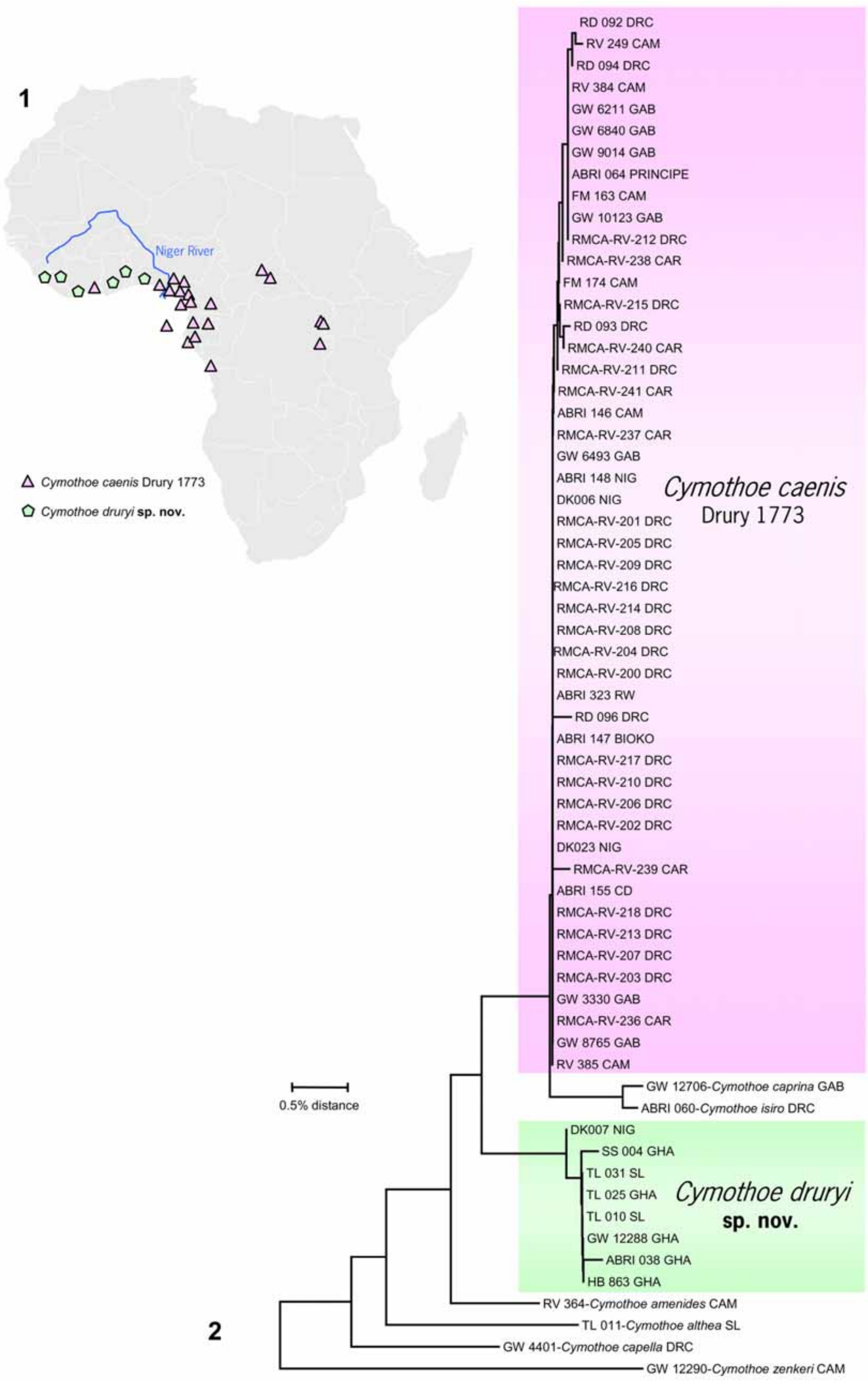

FIGURES 1–2. DNA barcoding results. 1. Map of Africa showing the collection sites for the specimens examined in this study. Sites where *C. caenis* s.s. and *C. druryi* **sp. nov.** were collected are represented by triangles and pentagons respectively. 2. Neighbor joining haplotype tree based on K2P distances between DNA barcodes. Each specimen is identified by its sample ID code (see Table 1). CAM = Cameroon, CAR = Central African Republic, DRC = Democratic Republic of the Congo, GAB = Gabon, GH = Ghana, GUI = Guinea, NIG = Nigeria, SL = Sierra Leone, RWA = Rwanda.

Materials and methods

We sampled DNA of 57 specimens of *Cymothoe caenis* s.l., including virtually the entire gamut of female forms, in addition to specimens of the sympatric *C. amenides* Hewitson, *C. althea* Cramer, *C. capella* Ward, *C. caprina* Aurivillius and *C. isiro* Rydon, which are considered to be related based on detailed observations of larval characters by Amiet (2000). *Cymothoe zenkeri* Richelmann, represented here by a single specimen, was used as outgroup as out of all *Cymothoe* sampled so far this species showed minimal K2P distance to the *C. amenides/caenis/capella/caprina/isiro* complex. Specimens were collected in Cameroon by van Velzen in 2006, and in Sierra Leone and Ghana by Larsen in 2006 and 2007, respectively. Additional specimens from Guinea, Ghana, Nigeria, Cameroon, Bioko, Principe, Gabon, Central African Republic, Congo DRC and Rwanda were kindly donated by collaborators (see Acknowledgements). See Table 1 for specimen details.

All specimens are vouchered and their metadata (i.e. taxonomy, collection, locality and voucher photographs) were submitted to the Barcoding of Life Database (BOLD; www.boldsystems.org) under the project name *CAEN*.

Legs, removed from dry, papered or mounted collection specimens, were processed at the Canadian Centre for DNA Barcoding (CCDB) where DNA was extracted using a routine silica-based 96-well extraction automated protocol (Ivanova *et al*., 2006). The target 658 base-pair fragment of the partial mitochondrial gene COI (DNA barcode) was amplified and sequenced as described by Hajibabaei and co-workers (2006). All sequences were deposited on GenBank (accession numbers GQ341626–Q341690)

We produced a haplotype tree based on DNA barcode distances using Neighbour Joining (NJ) in MEGA4 (Tamura *et al.*, 2007). Distance calculations were performed using the Kimura-2-parameter model (K2P: Kimura, 1980) including all sites and with pairwise deletion of missing data.

Available slides of male genitalia preparations of *C. caenis* s.l. were studied at the Natural History Museum in London. The preparations came from specimens originating in Sierra Leone [Rydon 102] (NHM, London 30414), Ivory Coast [Rydon 82] (NHM 30413), Nigeria (Calabar and Ikom) and Uganda, and were examined using light microscopy. These were subsequently compared with the male genitalia of DNA barcoded specimens from Cameroon [RV_249 and RV_385], Ghana [HB_863] (ZMA, Amsterdam RV1269), and Sierra Leone [TL_031] (ZMA RV1267), in addition to specimens from Guinea and Ivory Coast (Ph. Oremans coll.), and Nigeria (D.P. Knoop coll. and T. Pyrcz coll.).

Results

All samples yielded useful DNA from which the COI fragment could be obtained. The DNA barcode haplotype NJ tree (figures 1–2) separates the *C. caenis* s.l. sequences into two distinct clusters: 1) all sequences from specimens collected from the equatorial zone east of the Niger Delta in Nigeria (distance < 0.5%), and 2) those collected west of the Niger Delta (distance < 0.4%). Between these two clusters, the genetic differences are relatively large (distance 1.1%). Actually the eastern *C. caenis* haplotypes are genetically more similar to *C. caprina* and *C. isiro* (distance 0.8%) than they are to the western *C. caenis* haplotypes, with 1.1% distance (these two species are morphologically quite distinct from *C. caenis* s.l. and from each other). These results indicate that the eastern and the western haplotypes are reproductively isolated, and thus the existence of two cryptic species within *C. caenis* s.l. We emphasize, however, that DNA barcodes are strictly optimised for species identification and generally do not contain enough information for any thorough phylogenetic analysis. Moreover, distances do not necessarily reflect phylogenetic relationships. Therefore, the haplotype NJ tree does not allow phylogenetic statements and the sister relationships of the sampled species remain to be established.

Examination of male genital preparations revealed clear differences between specimens from western Africa (western Nigeria, Ghana, Ivory Coast and Sierra Leone) and from the equatorial zone (eastern Nigeria, Cameroon and Uganda), thus corroborating the results obtained from DNA barcodes. Figure 3 depicts male genitalia of the western type and the equatorial type.

TABLE 1. Details of the specimens from which DNA was extracted. L = sequence length; Dep. = depository collection (ABRI = African Butterfly Research Institute, DK = Dieuwko P. Knoop collection, GW = Gael Vande Weghe collection, RMCA = Royal Museum for Central Africa, ZMA = Zoological Museum Amsterdam); F = female; M = male. Sample ID is a unique identifier referring to the voucher specimen information in BOLD. Holotype of *Cymothoe druryi* **sp. nov.** is highlighted in bold characters.

Sample ID	L	Species	Dep.	sex	collector	Date Coll.	Country	Locality
TL_011	658	Cymothoe althea	ZMA	M	Larsen	Apr-2006	Sierra Leone	Gola Forest E
RV_364	658	Cymothoe amenides	ZMA	M	van Velzen	25-May-2006	Cameroon	Londji 2
RV_385	658	Cymothoe caenis	ZMA	M	van Velzen	Jun-2006	Cameroon	Djabilobe
RV_384	658	Cymothoe caenis	ZMA	F	van Velzen	Jun-2006	Cameroon	Djabilobe
FM_174	278	Cymothoe caenis	ZMA	F	Molleman	25-Aug-2004	Cameroon	Doumo Pierre
FM_163	658	Cymothoe caenis	ZMA	M	Molleman	18-Sep-2004	Cameroon	Doumo Pierre
RV_249.0	658	Cymothoe caenis	ZMA	M	van Velzen	05-May-2006	Cameroon	Malimba
ABRI_146	641	Cymothoe caenis	ABRI	F	Collins	Jan-2006	Cameroon	Manengouba, mt
RMCA-RV-236	658	Cymothoe caenis	RMCA	F	Hecq	02-Jun-1998	Central African Republic	Bohou river
RMCA-RV-241	630	Cymothoe caenis	RMCA	F	Hecq	Oct-1993	Central African Republic	Sangba reserve
RMCA-RV-240	249	Cymothoe caenis	RMCA	F	Hecq	Oct-1993	Central African Republic	Sangba reserve
RMCA-RV-239	658	Cymothoe caenis	RMCA	F	Hecq	11-Oct-1993	Central African Republic	Sangba reserve
RMCA-RV-238	250	Cymothoe caenis	RMCA	M	Hecq	Oct-1993	Central African Republic	Sangba reserve
RMCA-RV-237	650	Cymothoe caenis	RMCA	M	Hecq	Jan-1993	Central African Republic	Sangba reserve
RD_096	607	Cymothoe caenis	GW	F	Ducarme	06-Sep-2007	Congo DRC	Beni
ABRI_155	658	Cymothoe caenis	ABRI	M	unknown	Jan-2007	Congo DRC	Beni
RD_094	606	Cymothoe caenis	GW	M	Ducarme	27-Aug-2007	Congo DRC	Biakatu
RD_092	607	Cymothoe caenis	GW	F	Ducarme	07-Sep-2007	Congo DRC	Biakatu
RD_093	607	Cymothoe caenis	GW	F	Ducarme	16-May-2008	Congo DRC	Mamove
RMCA-RV-218	658	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-217	658	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-216	658	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-215	253	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-214	658	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-213	658	Cymothoe caenis	RMCA	M	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-212	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-211	278	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-210	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-209	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-208	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-207	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-206	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-205	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-204	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-203	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-202	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-201	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
RMCA-RV-200	658	Cymothoe caenis	RMCA	F	Desmet	30-May-2007	Congo DRC	Luki–Mayombe
ABRI_147	658	Cymothoe caenis	ABRI	M	Collins	Jan-2007	Equatorial Guinea	Bioko
GW_8765	658	Cymothoe caenis	GW	M	Vande Weghe	25-Feb-2006	Gabon	Akaka
GW_6211	658	Cymothoe caenis	GW	F	Vande Weghe	17-Dec-2004	Gabon	Ipassa

continued next page.

TABLE 1. (continued)

Sample ID	L	Species	Dep.	sex	collector	Date Coll.	Country	Locality
GW_3330	658	Cymothoe caenis	GW	F	Vande Weghe	07-Feb-2003	Gabon	Masuna2000
GW_9014	658	Cymothoe caenis	GW	M	Vande Weghe	17-Dec-2006	Gabon	Tchimbele
GW_10123	658	Cymothoe caenis	GW	M	Vande Weghe	21-Mar-2007	Gabon	Waka
GW_6840	658	Cymothoe caenis	GW	F	Vande Weghe	06-Feb-2005	Gabon	Waka
GW_6493	658	Cymothoe caenis	GW	F	Vande Weghe	02-Feb-2005	Gabon	Waka
DK023	658	Cymothoe caenis	DK	M	Knoop	Nov-2004	Nigeria	Oban N.P.
DK006	658	Cymothoe caenis	DK	F	Knoop	Nov-2004	Nigeria	Oban N.P.
ABRI_148	658	Cymothoe caenis	ABRI	M	Collins	Jan-2007	Nigeria	Obudu
ABRI_323	658	Cymothoe caenis	ABRI	M	Vingerhoedt	Jan-2007	Rwanda	Pindura
ABRI_064	658	Cymothoe caenis	ABRI	F	Collins	Jan-2008	Sao Tome and Principe	Principe
GW_4401	381	Cymothoe capella	GW	F	Vande Weghe	09-Jan-2004	Congo DRC	Bondo
GW_12706	658	Cymothoe caprina	GW	F	Vande Weghe	31-May-2007	Gabon	Waka
TL_025	658	Cymothoe druryi **sp. nov.**	ZMA	F	Larsen	Mar/Apr-2007	Ghana	Bobiri Forest
SS_004	658	Cymothoe druryi **sp. nov.**	ZMA	M	Szabolcs	13-18 May-2007	Ghana	Bobiri Forest
ABRI_038	658	Cymothoe druryi **sp. nov.**	ABRI	F	Vorgas	Jan-2005	Ghana	Kyabobo
HB_863	658	Cymothoe druryi **sp. nov.**	ZMA	M	Boersma	02-Dec-2007	Ghana	unknown
GW_12288	658	Cymothoe druryi **sp. nov.**	GW	M	Vingerhoedt	Dec-2005	Guinea	Diecké
DK007	264	Cymothoe druryi **sp. nov.**	DK	F	Knoop	Oct-1992	Nigeria	Agbara
TL_031	658	Cymothoe druryi **sp. nov.**	ZMA	M	Larsen	Apr-2006	Sierra Leone	Gola Forest E
TL_010	658	**Cymothoe druryi sp. nov.**	ZMA	M	Larsen	Apr-2006	Sierra Leone	Gola Forest E
ABRI_060	658	Cymothoe isiro	ABRI	M	Ducarme	Jan-2006	Congo DRC	Biakatu
GW_12290	658	Cymothoe zenkeri	GW	M	Vande Weghe	15-Dec-2004	Cameroon	Ebogo

Because the type locality of *Cymothoe caenis* is “Callabar” [Calabar] (Drury, 1773), situated east of the Niger Delta in the Cross River Loop, this is the valid name for the equatorial species. The synomym *C. adelina* Hewitson (1869) is from “Congo” and hence also equatorial, while all other names associated with *C. caenis* are invalid infrasubspecific names, also from the East. This necessitates a new name for the western species, which is proposed below. On 2 December 2008, van Velzen published the interim name “*Cymothoe caenis* nov.” on the BOLD website pending further research.

Cymothoe druryi van Velzen & Larsen sp. nov.

Figures 3–7

Description. Male (Figures 4–5). Forewing 32 mm long. The male is creamy white above with a hint of blueish-green. A faint straight discal line divides all wings in a slightly lighter basal half and a slightly darker distal half. There is a postdiscal line of irregular black spots, usually complete on the forewing, but incomplete on the hindwing. A dark, submarginal zig-zag band is separated from the equally dark margin. The abdominal fold is dark brown, and the basal part of both wings is dusted with grey which does not extend far into the wing surface. The underside is greenish-white with no tinge of yellow. A dark, fine, precise, straight discal line which is postdiscally lined with dark dustings crosses all wings. The submarginal chevrons appear as small, dark spots, connected by a light-grey submarginal band which is separated from the equally light-grey margin. The extent of the black markings varies broadly, but the pattern does not: variation is partly seasonally defined.

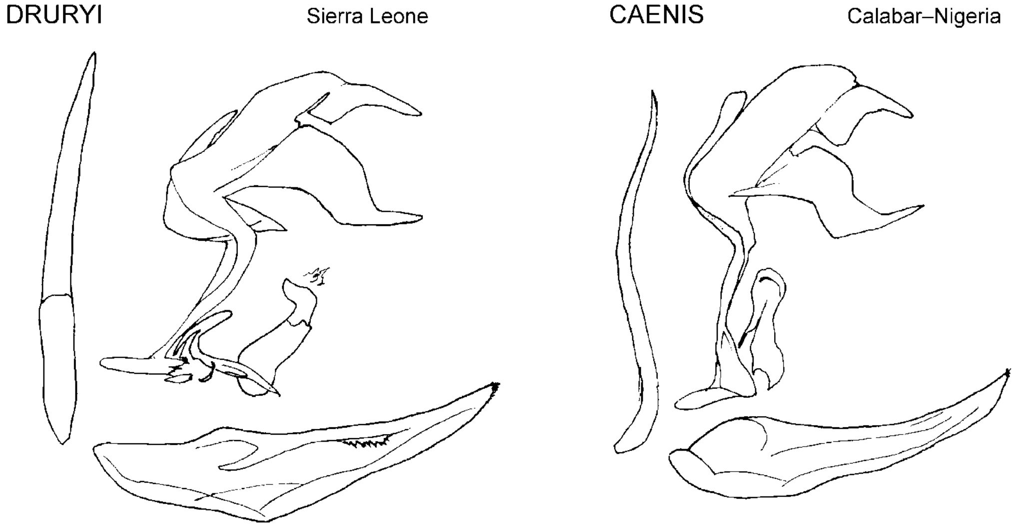

FIGURE 3. Male genitalia of *Cymothoe drury* **sp. nov.** from Sierra Leone [Rydon 102] (NHM, London 30414; the fultura is damaged) and *Cymothoe caenis* Drury from Nigeria [Rydon 81] (NHM, London 30446).

Female (Figures 6–7). Forewing 37 mm long. The upper side has the basal part of both wings dark-brown. This is followed by a white discal band that is precisely delineated, except at the end of the forewing cell where there are three irregular white spots. The band is so wide that the black postdiscal chevrons are largely inside the band. There is slight variation in the width of the white discal band, which is rarely (in <5% of the specimens) tinged with cream. The marginal chevrons are blackish-brown and bordered by a dark-brown margin. The underside shows the same pattern except for a fine, precise discal line bordering the dark basal part of the wings, and the submarginal chevrons which appear as small, dark spots, connected by a thin submarginal line.

Male genitalia (Figure 3). The uncus is 4 times as long as wide and of the same length as the ventral edge of the massive gnathos. The frontal part of the tegumen has a brief hump followed by a slight dome. The valves have a curved wedge-shape, with a long indentation at the dorsal edge anterior of the vinculum; the tips have teeth that continue briefly along the ventral edge of the valves. Below the dorsal edge of the valves is a well-defined, toothed harpe. The penis is of the same length as the valves and has a slight s-shape.

Material examined. Holotype: ♂ SIERRA LEONE, Gola Forest, April 2006 (T.B. Larsen leg., Zoological Museum Amsterdam coll.).

DNA barcoded paratypes: GUINEA: Diecké Forest, ♂ December 2006 (E. Vingerhoedt leg., G. Vande Weghe coll.); SIERRA LEONE: Gola Forest, ♂ April 2006 (T.B. Larsen leg., ZMA coll.); GHANA: Bobiri Forest, ♀ March/April 2007 (T.B. Larsen leg., ZMA coll.); Bobiri Forest, ♂ 13–18 May 2007 (S. Szabolcs leg., ZMA coll.); ♂ 2 December 2007 (J.H. Boersma leg., ZMA coll.); Kyabobo, ♀ June 2005 (R. Vorgas leg., ABRI coll.); NIGERIA: Agbara, ♀ October 1992 (D.P. Knoop leg. et coll.). All DNA barcoded types are referenced on the BOLD website.

Paratypes based on male genitalia: GUINEA: Diecké Forest, 3♂♂ 14–26 March 2003, 5♂♂ September 2003, 4♂♂ 7–16 May 2004 (Ph. Léonard & E. Vingerhoedt leg., Ph. Oremans coll.); IVORY COAST: Sassandra, ♂ October 1997 (Ph. Moretto leg., Ph Oremans coll.); NIGERIA: Isheri Forest, ♂ 10 March 1990 (D.P. Knoop leg., Muzeum Zoologiczne Uniwersytetu Jagiellonskiego coll.); Palm Grove Estate, Lagos, ♂ 22 October 1985 (A. Knoop leg., D.P Knoop coll.)

Etymology. The species is dedicated to Dru Drury who described *Cymothoe caenis* in his Illustrations of Natural History volume 2 (Drury, 1773), making it the first *Cymothoe* species known to science. Despite his major contribution to the taxonomy of West African butterflies, none carries his name. It had been the intention of Heinrich Neustetter, who described many forms of *Cymothoe* a century ago, to honour Drury with a species of *Cymothoe*, but it remained a manuscript name (Overlaet, 1952).

NB The type of *C. caenis* appears to have been lost: we shall designate a neotype, preferably from near the type locality in eastern Nigeria when more material is available.

Distribution. Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo and western Nigeria (see Figure 1).

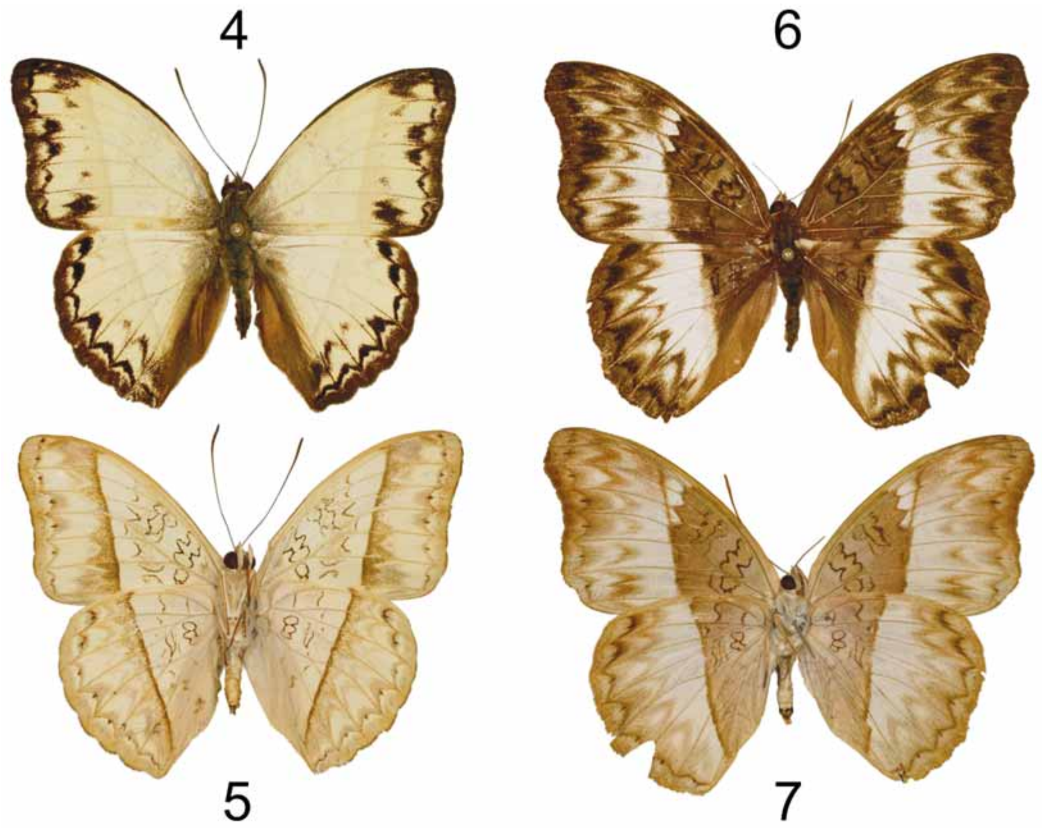

FIGURE 4–7. Photographs of *Cymothoe druryi* **sp. nov.** from Gola Forest in Sierra Leone. ♂ Holotype: 4. dorsal view; 5. ventral view. ♀ paratype: 6. dorsal view; 7 ventral view.

Diagnosis. The male is effectively identical to that of *C. caenis* Drury. No convincing differentiating macromorphological features were found despite the significant DNA differences reported above. The female is monomorphic but similar to one of the female forms of *C. caenis* that occur in the equatorial area.

The male genitalia of *C. druryi* differ significantly from those of *C. caenis* Drury in the following features: a) The uncus is distinctly longer, and b) the strongly chitinised gnathos is even more massive; the chitinised uncus has the same length as the ventral edge of the gnathos (that ratio is only 0.70 in *C. caenis*). c) The frontal part of the tegumen is much less strongly domed than in *C. caenis*. d) The dorsal edge of the valve, anterior of the vinculum, is indented in *C. druryi* and rounded in *C. caenis*. e) The tip of the valve has larger teeth and these are continued briefly along the ventral edge of the valve, whereas *C. caenis* has small teeth which are not continued. f) The most significant difference is the presence of a well-defined, toothed harpe below the dorsal edge of the valve which is absent in *C. caenis*. (see Figure 3).

Discussion

DNA barcoding

Since its inception in 2003 (Hebert *et al.*), DNA barcoding has proven to be an important tool in the discovery of cryptic species of Lepidoptera, for example in Costa Rica (Burns *et al.*, 2008; Hebert *et al.*, 2004;

Janzen *et al.*, 2005). These works addressed intraspecific variation on a local/regional scale representing only a portion of the species' distribution ranges; the emphasis was then made on species whose sympatry and genetic divergence indicated reproductive isolation. Indeed, many large-scale barcoding projects followed a region-based approach and critics have argued that this could cause an overestimation of the identification success of DNA barcoding (Frézal & Leblois, 2008; Moritz & Cicero, 2004; Will & Rubinoff, 2004). The basis for this concern is that local studies do not include closely related species that are not present in the area under study, causing an overestimation of interspecific distances, and, at the same time, underestimation of intraspecific DNA barcode variation (Elias *et al.*, 2007; Linares *et al.*, 2009; Meyer & Paulay, 2005). A comprehensive DNA barcoding study on Central Asian butterflies (Lukhtanov *et al.*, 2009) confirms these effects, but the authors also demonstrated that they did not affect accuracy of species discrimination.

In contrast, we followed a clade-based approach aiming at full taxonomic (and biogeographic) sampling. We have sampled specimens of *C. caenis* s.l. from its entire known geographical range. Additionally, we have included nearly all closely related species in our analysis (only *C. alticola* Libert & Collins and *C. consanguis* Aurivillius were not sampled) preventing an overestimation of interspecific distances. Reassuringly, DNA barcodes still discriminate species unambiguously with such extended geographic and taxonomic sampling. In addition, our results provide evidence for two cryptic species within *C. caenis* s.l. that are largely allopatric. Similar results were recently obtained for Neotropical sphingid and saturniid moths (Decaëns & Rougerie, 2008; Vaglia *et al.*, 2008) and for the two cryptic species *Cymothoe egesta* Cramer and *C. confusa* Aurivillius (McBride *et al.*, in press), suggesting the clade-based approach to be instrumental in uncovering hidden species that occur in allopatry.

Genitalia

The DNA barcoding results prompted careful examination of the male genitalia of *C. caenis* s.l. While assessing the status of the new species *Cymothoe alticola* Libert and Collins, Gérard Chovet compared its genitalia with those of *Cymothoe caenis* s.l. from West Africa (Ivory Coast) and from the equatorial zone (Cameroon/Congo DRC) reaching the conclusion that there were clear differences between all three (unpublished data). However, the issue of such differences in genital structure within *C. caenis* s.l. was never taken further (Libert, pers. comm.). As mentioned under Results, we have substantiated these differences, but closer study should yield additional characteristics. In relation to the present study, Dieuwko Knoop and Tomasz Pyrcz have confirmed that two sets of genitalia from Nigeria west of the Niger (from Palm Grove Estate, and Isheri Forest, respectively) match those of *C. druryi* (gen. DK-G101), further corroborating the match between differences in genitalia and DNA barcodes. Philippe Oremans further confirmed the genitalia identification of *C. druryi* males from Guinea and Ivory Coast.

Biogeography

Butterfly biogeographical patterns of West Africa are complex. Many strictly West African species do not cross the Volta River, while others – like *Cymothoe druryi* – do. Some of these species do not cross the Niger, others do. Some stop at the Cross River, others at the Sanaga River in Cameroon, while a few extend further to Gabon along the Cameroonian coast (*Acraea camaena* Drury, 1773 is the most outstanding example). The presence of such species in Nigeria, and further east, may stem from one of the several wetter interglacial periods during the Pleistocene or even the more recent Holocene when the Dahomey Gap, today an almost insurmountable savannah barrier for forest species, was well-forested (Adomou, 2005; Dupont & Weinelt, 1996; Maley, 1996; Salzmann & Hoelzmann, 2005). In addition, there are Nigerian/equatorial relicts in the Ghana/Togo mountains that never managed to cross the Volta River towards the west (*Larinopoda aspidos* Druce and *Euphaedra ruspina* Hewitson are excellent examples) (see Larsen, 2006).

Based on the presence of *C. druryi* in the Lagos area, we initially assumed that *C. caenis* s.s. and *C. druryi* were entirely allopatric, with the Niger River and Delta as boundary. This would be consistent with some of the biogeographical patterns outlined above, and may help shedding light on the distributional complexities of some other recently discovered butterflies of western Nigeria (Hecq & Larsen, 1997; Larsen, 2005). However, when Tomasz Pyrcz dissected Nigerian material in relation to the present study, he identified some specimens west of the Niger as *C. caenis* s.s. Surprisingly, he also found that two specimens from Banco Forest in Abidjan, Ivory Coast, deep inside the distribution of *C. druryi*, were also *C. caenis* s.s. These specimens carry the original labels from 1983, a year when Pyrcz was collecting only in Ivory Coast and Togo, and therefore we are confident about their locality data. *C. caenis* s.s. is known to be a long-distance migrant (e.g. Birket-Smith, 1960; Holland, 1892; Larsen, 2005; Schultze, 1917), which probably enabled it also to colonize e.g. Bioko and Principe and may explain its occurrence in Banco Forest. However, the Banco Forest *C. caenis* s.s. specimens are 1,150 km west of the closest Nigerian records, three times the distance between Principe and the *C. caenis* s.s. populations in Gabon.

Future prospects

Our brief description of *Cymothoe druryi* serves as an initial review in preparation for a more detailed account of both *C. druryi* and *C. caenis* and their relationships with other similar species, once more data are available. We would particularly like to answer the following questions:

1) What is the level of sympatry between the two species in Nigeria, and are there differences in host plant choice? *Cymothoe* species are highly host specific, and all species in the *C. caenis* group feed on members of the Achariaceae (ex Flacourtiaceae) (Amiet & Achoundong, 1996). In Ivory Coast, Vuattoux & Blandin (1979) have bred *C. caenis* s.l. on *Caloncoba gilgiana* (Sprague) Gilg, a small tree ranging in distribution from Sierra Leone to Nigeria (Hawthorne & Jongkind, 2006). This could indicate that the distribution of *C. druryi* matches that of its host plant. Likewise, *C. caenis* s.s. has been found on *Caloncoba welwitschii* Oliv. in Cameroon (Amiet & Achoundong, 1996), which occurs from Nigeria to Angola, Congo DRC and Tanzania (Hul, 1995); again in concordance with the butterfly's distribution. However, the same authors also discovered *C. caenis* s.s. feeding on *Caloncoba glauca* (P. Beauv.) Planch., which occurs throughout West and Central Africa (Hul, 1995).

2) Is there a genuinely resident population of *C. caenis* s.s. in the Banco Forest, or do they represent the progeny of migrants that since went extinct? Analysis of DNA sequences from the Banco Forest specimens may give indications about their origin and gene-flow, and increased sampling within western Africa might reveal additional populations of *C. caenis* s.s. in that area.

3) Can we recognise a geographical pattern in the multitude of female forms in *C. caenis*? Many of these forms can occur in sympatry, but some forms may be limited to a particular area. In addition, some populations appear to be morphologically more diverse than others, and geographical patterns of variability may give insight into the ecological causes of the species' polymorphism.

4) Can sufficient data be found to analyse the migration pattern of *C. caenis*? So far, mass migratory behaviour has been described for central African populations only, indicating that this is a specific character of *C. caenis* s.s.; but its ecology remains poorly understood. We would like to examine whether meteorological or demographic factors play a role in triggering this behaviour.

In conclusion, our results as well as those of others (Hecq & Larsen, 1997; Van Velzen et al., unpub.) indicate that the Niger Delta and western Nigeria may have played an important role in butterfly speciation, both as a rain forest refuge area and as a dispersal barrier. Tomasz Pyrcz and Torben Larsen are preparing a meta-analysis of the biogeographical complexities of butterflies in the Niger Delta and western Nigeria.

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