

***ATROCALOPTERYX MELLI OROHAINANI* SSP. NOV.
ON THE ISLAND OF HAINAN, CHINA
(ZYGOPTERA: CALOPTERYGIDAE)**

Z. GUAN, B.-P. HAN* and H.J. DUMONT*
Institute of Hydrobiology, Jinan University, Guangzhou-510632, China

Received December 20, 2011 / Revised and Accepted December 30, 2011

The new sp. is described from the mountain core of Hainan, southern China, where it usually occurs at altitudes not lower than 300 m asl. It lives on the same type of small, shaded rivers as the nominate ssp. on the continent, and is distinguished by its larger size, slightly less enfumed wings, and a 2.6% difference in the sequence of the barcoding portion of the mitochondrial DNA-cytochrome c oxidase subunit I gene (COI). Holotype ♂: Diaoluoshan mountain, 6-VIII-2011; deposited in the Inst. Hydrobiol., Jinan Univ., Guangzhou. It is argued that this geographically defined ssp. evolved because of persistent poor gene flow with continental populations, caused by the lowland “panhandle” between Hainan and the continent. This barrier was probably functioning equally well during interglacials (like at present) as during pleniglacials (when Hainan was connected to the mainland), because lack of suitable environments (small sized running waters), and dry and cold conditions continued to limit the contact with *A. melli* of the mainland.

INTRODUCTION

The island of Hainan, the southernmost province of China, is well known for its tropical climate, high biodiversity, and high level of endemism in different animal and plant groups. The number of odonate species known from the island currently amounts to 165. Including the taxon described hereinafter, there are 23 endemics (REELS & ZHANG, 2012), with *Pseudolestes mirabilis*, the sole representative of the family Pseudolestidae, the flagship species.

The island is currently separated from the mainland by an 18 km wide sea-strait that became exposed during the most recent pleniglacial, in principle opening up the island to colonization from the mainland. The core of the island is a granit-

* Corresponding authors: henri.dumont@ugent.be; – tbphan@jnu.edu.cn

ic mountainous region that represents the geologically oldest part of China. It peaks at 1867 m asl (Wuzhishan mountain), and is covered in tropical rainforest, from which numerous streamlets descend to a number of larger rivers.

Here, we discuss the representative of *Calopteryx melli* that lives on these streamlets, generally beginning around 300 m and with the upper limit still ill-defined. In all, populations are now known from 3 such localities (WILSON & REELS, 2001). It mainly stands out by its size, the significance of which is here tested by sequencing a fragment of a mitochondrial gene (COI) in a comparison involving also several mainland populations. It was originally described by RIS (1912) from Guangdong (“Kwan-Tung”), in a locality named “Tsa Yiu San” (San almost certainly standing for Shan, hill or mountain) and has since been found to occur in several (see below) southern provinces of China. The reasons for moving this

Table I
The eight populations of *Atrocalopteryx melli* included in this study

Date	Site	GPS / Altitude	Location in China
8-VI-2011 24-VII-2011	Dinghushan mountain, Zhao Qing city, Guang- dong province	N 23°10'21"; E 112°31'39" / 200 m	South, Continent
6-VIII-2011	Diaoluoshan mountain, Hainan	N 18°43'30.76"; E 109°52'06.12" / 908 m	South Island
8-VIII-2011	Sanyatang mountain, Cong Hua City, Guang- dong	N 23° 45' 50.33"; E 113°49'1.15" / 600 m	South, Continent
9-VIII-2011	Chebaling Nature Reserve, Shao Guan City, Guangdong	N 24°40'29"; E 114°09'04" / 450 m	South, Continent
12-VIII-2011	Tian men shan mountain, Yongtai county, Fujian	N 25°49'17.15"; E 119°00'45.20" / 210 m	East, Continent
18-VIII-2011	Lingui Country (near Huaping National Reserve), Guilin City, Guangxi	N 25°32'11.99"; E 110°00'28.23" / 503 m	West, Continent
19-VIII-2011	Qingshitan mountain, Lingchuan County, Guilin City, Guangxi	N 25°30'52.70"; E 110°13'21.05" / 208 m	West, Continent
23-VIII-2011	Baiyunyan mountain, Longquan County, Lishui City, Zhejiang	N 28°06'11.97"; E 119°05'38.45" / 103 m	East-north, Continent

species to the genus *Atrocalopteryx*, created by DUMONT et al. (2007), are found in a paper (GUAN et al., 2012) updating the phylogeny of the Calopteryginae.

MATERIAL AND METHODS

Most material was collected in summer 2011, although visits to the provinces of Guangdong, Guangxi, Fujian, Zhejiang and Hainan had been made since 2008. Animals were collected by netting and preserved in 70% alcohol in the field. In the laboratory, they were stored in a refrigerator until further study, which consisted of measuring the abdomen, wing length, and size of the apical wing spot (a diagnostic feature of the species), and removing pieces of thoracic muscle for DNA extraction.

Table I provides an overview of the populations included in this study, their geographic origin, and the dates of sampling.

DNA studies involved the isolation and sequencing of the barcoding portion of the mitochondrial COI gene, which has been shown to be a marker for relatively shallow phylogenetic relationships (roughly from subspecies to family) in the Odonata. For detailed procedures, from DNA extraction to gene alignments and mathematical manipulations, see BYBEE et al. (2008), DUMONT et al. (2005, 2007, 2010) and WEEKERS et al. (2001). In all, four specimens from four populations were analyzed (see Fig. 2 for origin of these populations).

RESULTS

Figure 1 gives a maximum likelihood estimate of the relationship between the different *melli*-populations, rooted against *Atrocalopteryx oberthueri* as an outgroup. The result convincingly shows three continental populations to cluster closely together, with minimal sequence variation (the specimens sequenced contained only three haplotypes and differed by maximum two base pairs). The island population, in contrast, stood out with an average of 2.6% sequence difference (19 base substitutions) with the mainland ones.

Tables IIa and b show the result of the measurements and of pairwise t-tests with exact

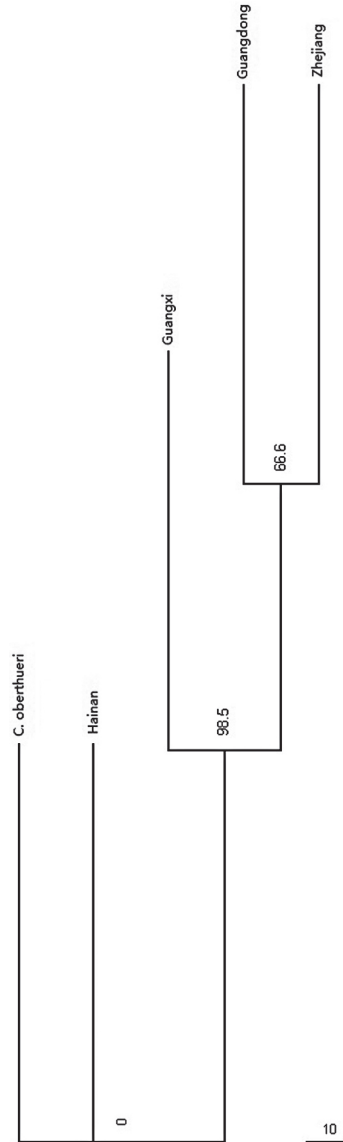


Fig. 1. Estimate of the phylogenetic tree of *Atrocalopteryx melli* (maximum likelihood method, 100 bootstraps, 10 replicas), using *A. oberthueri* from Sichuan, China, as an outgroup.

Table IIa
Values of body lengths in eight *A. melli* populations, means \pm SE (cm)

		Body length	Wing width	Wing length	Wing spot
Male	Late Season (n=15)	6.95 \pm 0.14	1.44 \pm 0.06	4.61 \pm 0.11	0.73 \pm 0.02
	Mid-Season (n=21)	7.39 \pm 0.09	1.43 \pm 0.03	4.73 \pm 0.06	0.81 \pm 0.02
	Hainan (n=6)	7.83 \pm 0.09	1.53 \pm 0.03	5.02 \pm 0.05	0.83 \pm 0.04
	Mainland (n=30)	7.10 \pm 0.08	1.41 \pm 0.03	4.62 \pm 0.06	0.77 \pm 0.02
Female	Late Season (n=3)	6.38 \pm 0.12	1.53 \pm 0.03	4.47 \pm 0.03	0.90 \pm 0.10
	Mid-Season (n=7)	6.76 \pm 0.16	1.49 \pm 0.02	4.87 \pm 0.09	0.87 \pm 0.02
	Hainan (n=0)	—	—	—	—
	Mainland (n=10)	6.65 \pm 0.13	1.51 \pm 0.02	4.75 \pm 0.09	0.88 \pm 0.03

probabilities of body lengths, wing lengths and maximum width, and size (length) of the apical wing spot in eight populations. It was found that Hainan animals are consistently and significantly taller and have broader wings than mainland ones, but they do not differ in apical wing spot size. The latter is a secondary sexual character, however, and was found to be significantly bigger in females than males.

Atrocalopteryx melli is a species with a long flight period, extending from May till October (with maximum in August), and an effect of season on size and wing spot was also noted.

ATROCALOPTERYX MELLI OROHAINANI SSP. NOV.

Figures 1-2

Material. — **Holotype** ♂: China, Hainan, Diaoluoshan mountain, 6-VIII-2011; deposited (in 70% ethanol) in Inst. Hydrobiol., Jinan Univ., Guangzhou; — **Paratypes** 2♂, same data, deposited in H.J. Dumont collection.

Etymology. — The name is a composition of the Greek noun “oros” (= “mountain”), combined with the name of the island, in order to bring out the fact that the animal is restricted to the mountain zone of Hainan.

DESCRIPTION. — Body entirely metallic green, darkening towards tip of abdomen. Legs black. No postocular tubercles. Face metallic green, labrum black with

Table IIb
Pairwise *t*-tests of body measurements in eight *A. melli* populations, with exact P values. Significant probabilities are marked in bold

	Body length	Wing width	Wing length	Wing spot size
Hainan vs Mainland	-4.387 / 0.007	-7.445 / 0.001	-5.678 / 0.002	-1.195 / 0.286
Female vs Male	0.634 / 0.542	-2.788 / 0.021	-1.497 / 0.168	-3.737 / 0.005
Early vs Late Season	-2.473 / 0.025	-0.272 / 0.789	-2.014 / 0.061	-2.252 / 0.039

two lateral yellow patches. Ligula as in *Calopteryx*. Superior appendices forcipate, *Calopteryx*-like; inferiors straight, markedly shorter than superiors. Wing venation typical for *Atrocalopteryx*, wing apex deep brown, venation brownish, darkened along costal margin of wing but no or indistinct darkened central zone on wings. Total length of holotype 7.9 cm, abdomen 6.6 cm, forewing 5.1 cm.

DIFFERENCES WITH *ATROCALOPTERYX M. MELLI* (RIS, 1912). — Morphologically, and apart from the larger size of the insular taxon, no differences could be found, but Hainan animals had clearer wings than mainland ones (Fig. 2). The arrow on the figure indicates a patch of brown colour on the male wings, roughly below the nodus, that is less well expressed in Hainan animals.

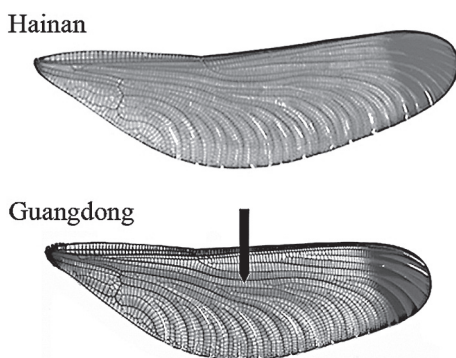


Figure 2. A comparison of the forewings of an insular and a continental *Atrocalopteryx melli* population. The somewhat fuzzy brown spot close to the centre of the wing surface in the continental population is arrowed.

DISCUSSION

The above results lead us to conclude that the Hainan population is best ranked as a geographic subspecies to *Atrocalopteryx melli*. *A. m. orohainani* ssp. n. is restricted to the mountain zone of Hainan, where it probably has no genetic contact with other, continental populations. This isolation, emphasized by its specific DNA, is the most important argument to raise it to the level of an endemic taxon. The other characters (size, wing colour), taken alone, could be considered debatable evidence upon which to base such a decision.

The 2.6% base difference in the COI is tacitly assumed to be representative for the whole genome. This level of difference strongly suggests that the age of the subspecies is much older than the last glacial episode, and probably goes back to the early Pleistocene. During interglacials, rising sea-levels repeatedly created a sea strait between Hainan and the continent, preventing any contact with the mainland, while during pleniglacials, the island would become a peninsula, but colder and drier conditions than today would again prevent the dispersal of such rheophilic organisms across a dry lowland between the mountains of Hainan and the hills of Guangdong province. Consequently, the origin of *A. melli orohainani* must be sought in the generally warm and humid climate during the transition period between the Pliocene and Pleistocene.

ACKNOWLEDGEMENTS

Support by the grant for leading talent scientists of Guangdong Province to Dr Henri Dumont and an innovative grant to Dr Zhaoying Guan in the Hydrobiology Program of 211 Project at Jinan University is appreciated. Our thanks to Dr MARTIN SCHORR for kindly providing a pdf of Ris' original description of *Calopteryx melli*.

REFERENCES

- BYBEE, S.M., T.H. OGDEN, M.A. BRANHAM & M.F. WHITING, 2008. Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics* 23: 1-38.
- DUMONT, H.J., J.R. VANFLETEREN, J.F. DE JONCKHEERE & P.H. WEEKERS, 2005. Phylogenetic relationships, divergence time estimation, and global biogeographic patterns of Calopterygoid damselflies (Odonata, Zygoptera) inferred from ribosomal DNA sequences. *Syst. Biol.* 54: 347-362.
- DUMONT, H.J., A. VIERSTRAETE & J.F. VANFLETEREN, 2007. A revised molecular phylogeny of the Calopteryginae (Zygoptera: Calopterygidae). *Odonatologica* 36: 365-372.
- DUMONT, H.J., A. VIERSTRAETE & J.R. VANFLETEREN, 2010. A molecular phylogeny of the Odonata (Insecta). *Syst. Ent.* 35: 6-18.
- GUAN, Z., B-P HAN, A. VIERSTRAETE & H.J. DUMONT, 2012. Additions and refinements to the molecular phylogeny of the Calopteryginae s.l. (Zygoptera: Calopterygidae). *Odonatologica* 41: 17-24.
- REELS, G.T. & H.M. ZHANG, 2012. *A field guide to the dragonflies of Hainan*. Kadoorie Farm & Botanic Garden & China Forestry Publishing House, Beijing. — [in press]
- RIS, F., 1912. Neue Libellen von Formosa, Südchina, Tonkin und dem Philippinen. *Supplta Ent.* 1: 44-85.
- WEEKERS, P.H.H., J.F. DE JONCKHEERE & H.J. DUMONT, 2001. Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representatives of the genus *Calopteryx* (Insecta: Odonata) of the West Mediterranean and adjacent West European zone. *Mol. Phylogen. Evol.* 20: 89-99.
- WILSON, K.D.P. & G.T. REELS, 2001. Odonata of Hainan, China. *Odonatologica* 30: 145-208.