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New and old data support the specific separation of *Aporia kanekoi* Koiwaya, 1989 from *Aporia larraldei* (Oberthür, 1876)

(Lepidoptera, Pieridae)
by

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Abstract: Field and molecular evidences confirm that *Aporia larraldei* (Oberthür, 1876) and *Aporia kanekoi* Koiwaya, 1989 are distinct species. In particular the latter has been found syntopic and synchronic with *A. larraldei shizuyai* Koiwaya, 1996 near Wenchuan, Sichuan, China. Genetic data about the *Aporia* taxa object of this study, obtained from COI mitochondrial DNA investigation, are also given: they equally strongly support the specific separation of *A. larraldei* (Obth.) and *A. kanekoi* Koiwaya.

Within the genus *Aporia* Hübner, [1819], the taxa of the *larraldei* complex constitute a homogeneous group. In chronological order of description they are: *larraldei* (Oberthür, 1876), *melania* (Oberthür, 1892), *nutans* (Oberthür, 1892), *kanekoi* Koiwaya, 1989, *shizuyai* Koiwaya, 1996, *kaolinkonensis* Yoshino, 1997 and *caofengi* Huang, 2021. A common feature distinguishes them: in the ♂ genitalia the uncus shows a particular spatulate shape, evident above all in the dorsal view, with the rounded end and slightly incised in the centre.

In a recent paper Huang (2021) has proposed a systematic ordering differing from that followed by Della Bruna et al. (2013) who recognized the existence of two species: *A. larraldei* (Obth.), including the subspecies *melania* (Obth.)*, nutans* (Obth.)*, shizuyai* Koiwaya and *kaolinkonensis* Yoshino, and the monotypic *A. kanekoi* Koiwaya.

In essence, Huang elevated the taxon *melania* (Obth.) to the rank of good species, aggregating to it the taxa *nutans* (Obth.) (= *kaolinkonensis* Yoshino) and *caofengi* Huang as subspecies. He also downgraded *A.kanekoi* Koiwaya to a subspecies of *A. larraldei* (Obth.) pointing out that the cases of sympatry reported in the literature as support of their specific separation (Koiwaya, 1989, Della Bruna et al*.*, 2013) would not be conclusive as they were actually found between *A. kanekoi* Koiwaya and *A. melania* (Obth.), hypothesizing the latter as a distinct species from *A. larraldei* (Obth.).

Apparently, Huang proposed his taxonomic conclusions based solely on morphology and his knowledge of the geographical distribution of these taxa under study.

In this brief note we present some data on the geographical distribution and on the genetic relationships of the studied taxa which remained little known or completely ignored for a long time. On the basis of these data we will demonstrate that Huang's taxonomic conclusions are untenable and that the correct systematic arrangement of the *A. larraldei* (Obth.) group is just the one outlined in our previous review.

First of all, the sympatric occurrence of *A. kanekoi* Koiwaya and *A. larraldei* (Obth.) is no more questionable. Indeed we found *A. kanekoi* Koiwaya, and *A. larraldei shizuyai* Koiwaya, flying together in a valley in Sichuan, between Guan Xian and Wenchuan, about 30 km south of the latter locality at about 1300 m of altitude (31°13'45 "N–103°28'15"E). These records dating back to 24 and 26 June 2005 clearly account for not only sympatry yet also syntopic occurrence of *A. larraldei* (Obth.) and *A. kanekoi* Koiwaya, and, interestingly, represent the northernmost locality for *A. kanekoi* Koiwaya, shifting further north the known range distribution boundary of this species.

In all, 29 ♂♂ of *kanekoi* and 3 ♂♂ of *A. l. shizuyai* Koiwaya, were caught; furthermore, on a previous trip in July 1994, one of us (CDB) had already found a ♀ of *A. kanekoi* Koiwaya near Wenchuan. The numerical disproportion found may suggest a greater rarity of *A. l. shizuyai* Koiwaya in the aforementioned locality or a different phenology of the two taxa, both shown here (Figs. 1-2).

As can be seen from the photos *A. kanekoi* is appreciably larger with clearly more marked black designs: in particular, as already noted by Huang, on the upper page of the rear wing the cubital vein is always blackened while it is not at all in *A.l. shizuyai* Koiwaya. Even the ♂ genitalia of the two taxa, although very similar, are not entirely identical. In *shizuyai* the valve appears more slender and pointed, the saccus is shorter and, in dorsal view, shows the maximum width at about one third of its length, narrowing towards the apex. On the contrary, in *kanekoi* the saccus has a constant width from the base to the tip and, in lateral view, a more wavy shape.

Moreover, there is even more strong evidence that *A. larraldei* (Obth.) and *A.kanekoi* Koiwaya are distinct species from the genetic point of view. Actually, from the molecular analyses carried out on COI sequences, conducted more than ten years ago in the laboratory of one of us (VS), it had already emerged that the two species are sister taxa and that the level of genetic divergence between them is largely above of the values generally accepted to separate two species.

Table 1 shows the pairwise genetic distance values calculated between *Aporia* taxa according to Kimura two-parameter (K2P) model (Kimura,1980) on COI mitochondrial DNA sequences.

Indeed, analyzing sympatric and syntopic individuals of the two taxa, the genetic distance between all the samples of *A.larraldei* (Obth.), including *A.l. shizuyai* Koiwaya, and two individuals of *A.kanekoi* Koiwaya was always equal to or greater than 0.05 (Deodati, 2010).

Genetic and phylogenetic relationships between *Aporia* taxa object of this study are also reported in Fig. 3.

In detail, the examined specimens were labelled as follows*: A. l. shizuyai* Koiwaya, 1 ♂, Sichuan, Wenchuan-Dujiangyan, 30 km S Wenchuan, 1300 m, 31°13'45"N – 103°28'15"E, 26 .VI.2005, E. Giacomazzo legit and *A. kanekoi* Koiwaya, 1 ♂, same data as above and 1 ♂, Sichuan, Dayi District, 1150-1350 m, 30°40'02"N – 103°15'11"E, 29.VI.2006 , V. Sbordoni legit.

On that occasion, samples of *A. l. larraldei* (Obth.) and *A.l. melania* (Obth.) were also analysed, and also for this last taxon the results obtained contradict Huang's idea since the genetic distance values between three specimens of *A. l. melania* (Obth.), one of *A. l. larraldei* (Obth.) and the other one of *A. l. shizuyai* Koiwaya were below 1%, therefore confirming the ranking of the taxon *melania* (Obth.) as a subspecies of *A. larraldei* (Obth.).

Generally speaking, the use of appropriate DNA sequences such as DNA barcoding has produced important advances in our ability to discriminate taxa at the species level. Although DNA barcoding sequences are not always adequate to capture the evolutionary divergence, as in the case of recent speciation events, the use of this tool has revolutionized the way of doing taxonomy and should not be ignored in the era of integrative taxonomy or multidimensional systematics (Sbordoni, 2010). Moreover, the fine tuning of the levels of genetic divergence offers valuable insights in the species geographical variation and its meaning in speciation.

Summarizing all the above, it is evident that taxa *kanekoi* Koiwaya and *shizuyai* Koiwaya, which are furthermore sympatric, belong to two different species while *shizuyai* Koiwaya, *melania* (Obth.) and *larraldei* (Obth.) are conspecific. For the other taxa of the group, namely *nutans* (Obth.), *kaolinkonensis* Yoshino and *caofengi* Huang, molecular data are not available at present. However, on the basis of their reciprocal resemblance and considering that they are very similar to *A. melania* (Obth.), we can reasonably suppose that all belong to *A. larraldei* (Obth.).

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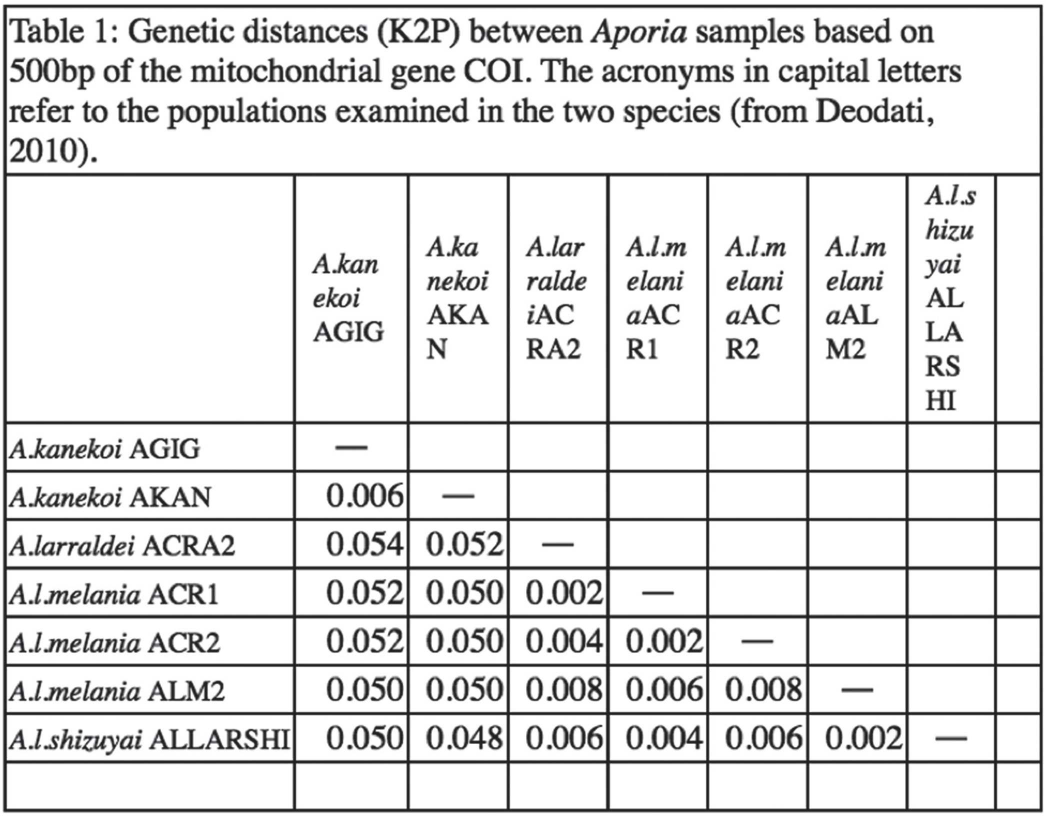

Table 1: Genetic distances (K2P) between *Aporia* samples based on 500bp of the mitochondrial gene COI. The acronyms in capital letters refer to the populations examined in the two species (from Deodati, 2010).

	A.kanekoi AGIG	*A.kanekoi* AKAN	*A.larraldei* ACRA2	*A.l.melania* ACR1	*A.l.melania* ACR2	*A.l.melania* ALM2	*A.l.shizuyai* ALLARSHI	
A.kanekoi AGIG	—							
A.kanekoi AKAN	0.006	—						
A.larraldei ACRA2	0.054	0.052	—					
A.l.melania ACR1	0.052	0.050	0.002	—				
A.l.melania ACR2	0.052	0.050	0.004	0.002	—			
A.l.melania ALM2	0.050	0.050	0.008	0.006	0.008	—		
A.l.shizuyai ALLARSHI	0.050	0.048	0.006	0.004	0.006	0.002	—	

Fig. 1: Habitus of *Aporia larraldei shizuyai* Koiwaya, 1996 ♂ (above) and *Aporia kanekoi* Koiwaya, 1989 ♂ (below), sympatric and synchronic, upperside. CHINA, Sichuan, 30 km S of Wenchuan, m 1300, 26.VI.2005, leg. et coll. E. Gallo (scale bar = 1 cm).
Fig. 2: Underside of the same specimens.

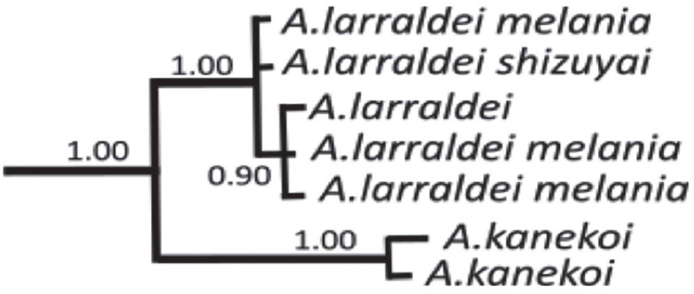

Fig. 3: Bayes consensus tree showing genetic relationship between *Aporia larraldei* (Oberthür, 1876) and *Aporia kanekoi* Koiwaya, 1989 based on 500bp of the mitochondrial gene COI (modified from Deodati, 2010).

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