Zootaxa 1691: 67–68 (2008)
www.mapress.com/zootaxa/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Using DNA barcodes to assess identity and diversity of *Dendropsophus minutus:* Failure?

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The 5' end (Folmer or Barcode region) of cytochrome *c* oxidase 1 (CO1) has been proposed as the gene region of choice for a standardized animal DNA barcode (Hebert et al. 2003). Concerns have been raised regarding the decision to utilize this particular mitochondrial gene region as a barcode. Nevertheless, widely divergent taxonomic groups have reported success using CO1 for both species identification and discovery. The utility of CO1 for barcoding amphibians was raised early on (Vences, et al. 2005) and concerns for this group were reported widely (Waugh 2007)—although some considered that the reporting of the concerns outstripped the data that had been analyzed at that point (Smith et al. 2008). Indeed, our analysis of CO1 for a small group of Holarctic amphibians was neither more difficult to generate nor to analyze than for other groups where we have utilized the technique.

Hawkins et al. (2007) present CO1 data for 73 individuals from a species complex of *Dendropsophus minutus* from the Guianas. This group has been in a known state of taxonomic uncertainty since its description in 1872—and hypotheses regarding the prevalence of cryptic species have been made. It appears to be an interesting test case for the utility of the CO1 DNA barcode for amphibian identification.

Hawkins et al. report that their data supports, '*previous assertions that the conventional DNA barcode locus is not suitable for amphibians'*—p. 61. This support appears to be based on the observation that they, "*… encountered a number of problems in our attempts to amplify the universal barcode locus for this taxon that necessitated the development of a novel primer. Furthermore, a number of individuals for which we have samples from the Amazon basin (not included in this study) would not amplify for CO1 (though we have been able to amplify nuclear DNA from these samples).*'—p. 64.

I have two concerns regarding the presentation and interpretation of their data. One is their proposition that their data represents the "conventional DNA barcode locus", while the other is the use of their data to conclude a failure of the locus or the approach.

Hawkins et al. propose that their dataset is "perfect" for the analysis of the utility of CO1 barcoding with amphibians. However, there is a maximum of 163 bp overlap between their sequence with the conventional DNA barcode region and so the data does not meet all the Genbank and Consortium for the Barcode of Life (CBOL) specifications for animal BARCODE standards (Hanner 2005). It has been shown that shorter than standard barcode reads often contain enough information for accurate species identification (Hajibabaei et al. 2006). Indeed, when the Hawkins et al. sequences are reduced to the 160 bp of the barcode region—the truncated data maintains the predictions of their 700 bp analysis (namely D is very divergent from ABC—and ABC have minimal divergence that would require further ecological, morphological, acoustic etc. information prior to the erection of any species-level hypotheses (Figure 1)). This very short portion of the barcode region has succeeded in identifying a provisional cryptic species.

The authors themselves used the deeply divergent CO1 sequence from lineage "D" to hypothesize that this lineage represents an, as yet morphologically and acoustically, cryptic species. *"The lineage here referred to as group D is highly divergent from the ABC lineage and likely represents a distinct, cryptic species."*—p. 64. I would argue that the erection of such a falsifiable hypothesis using partial CO1 barcode data should constitute a successful use of CO1 barcoding with amphibians rather than as the authors appear to have interpreted it; as a failure.

The region has allowed the authors to make hypotheses regarding further cryptic speciation, so the apparent failure of the method involves recalcitrant amplification. Unfortunately, the problems of amplification are not reported in any detail. This absence prevents any interested researcher/laboratory from exploring, considering or replicating their methodology. What primers were utilized that failed? What were the amplification conditions? The authors present the absence of information as proof of support for the hypothesis that the conventional DNA barcode locus is not suitable for amphibians. We have amplified CO1 from nearly 40 genera from 11 families using only previously published primer pairs originally designed for other vertebrates, or insects (Smith et al 2008). The issue of lack of amplification success,

Accepted by M. Vences: 20 Dec. 2007; published: 29 Jan. 2008

when not supported by data, does not contain sufficient weight to be considered as supporting prior assertions that amphibians are a problem group for the animal DNA barcode region of CO1.

Undoubtedly, and no differently to other taxa, there may be problems with CO1 as a single gene region DNA barcode for some groups of amphibians. However, Hawkins et al. data is neither complete nor specific enough to warrant the conclusion that it supports the lack of utility for the CO1 barcode locus for amphibians. Their sequences have only a restricted region of overlap with the barcode region—and even so an analysis of that overlap supports the affirmation of cryptic provisional species (Group D). I would argue that this is a success of the CO1 methodology. It appears that this is a case where amphibians have been presented as a problem group for CO1 DNA barcoding in advance of a thorough test of the proposition. A better test would include an analysis and presentation of specifically where and how the CO1 approach failed. I predict that the growing standard array of published CO1 primers and methodologies should allow more lab groups to systematically test of the utility of a CO1 DNA barcode for amphibians.

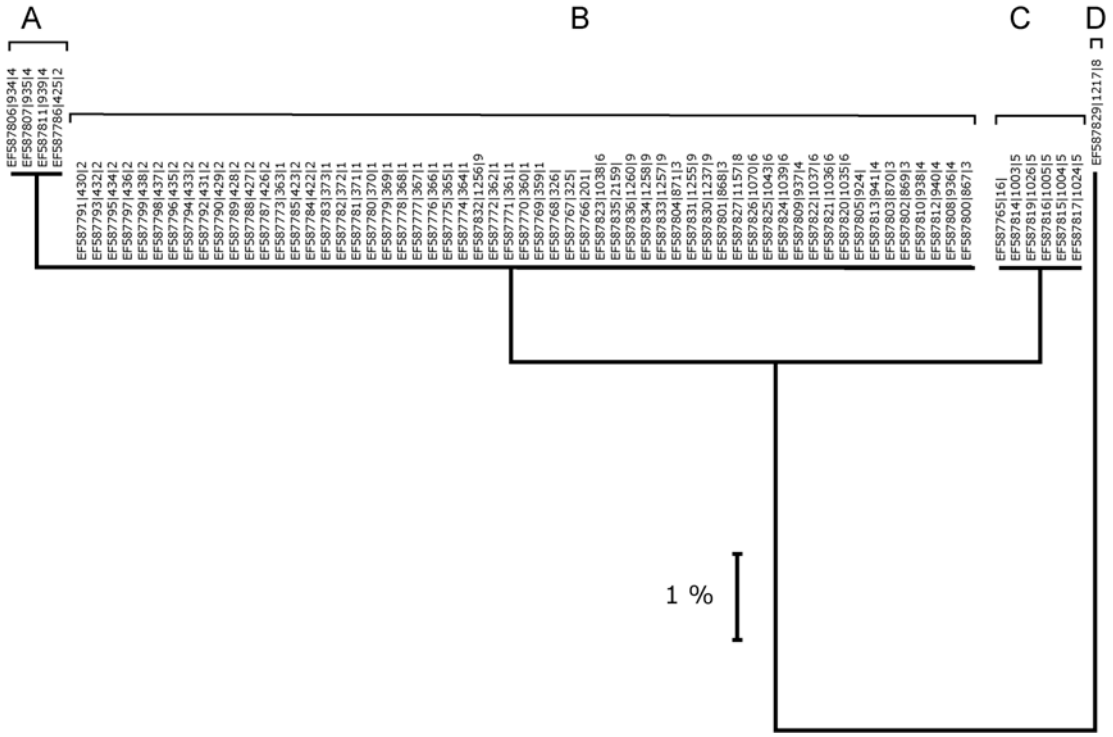

FIGURE 1. Hawkins et al. CO1 dataset reduced to the 163 bp that overlaps with the conventional DNA barcode region. Branch tips are labeled with Genbank accession numbers, collection accessions and population coding. See www.barcodinglife.org, Published Projects, *Dendropsophus minutus* CO1: Failure or Success?

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