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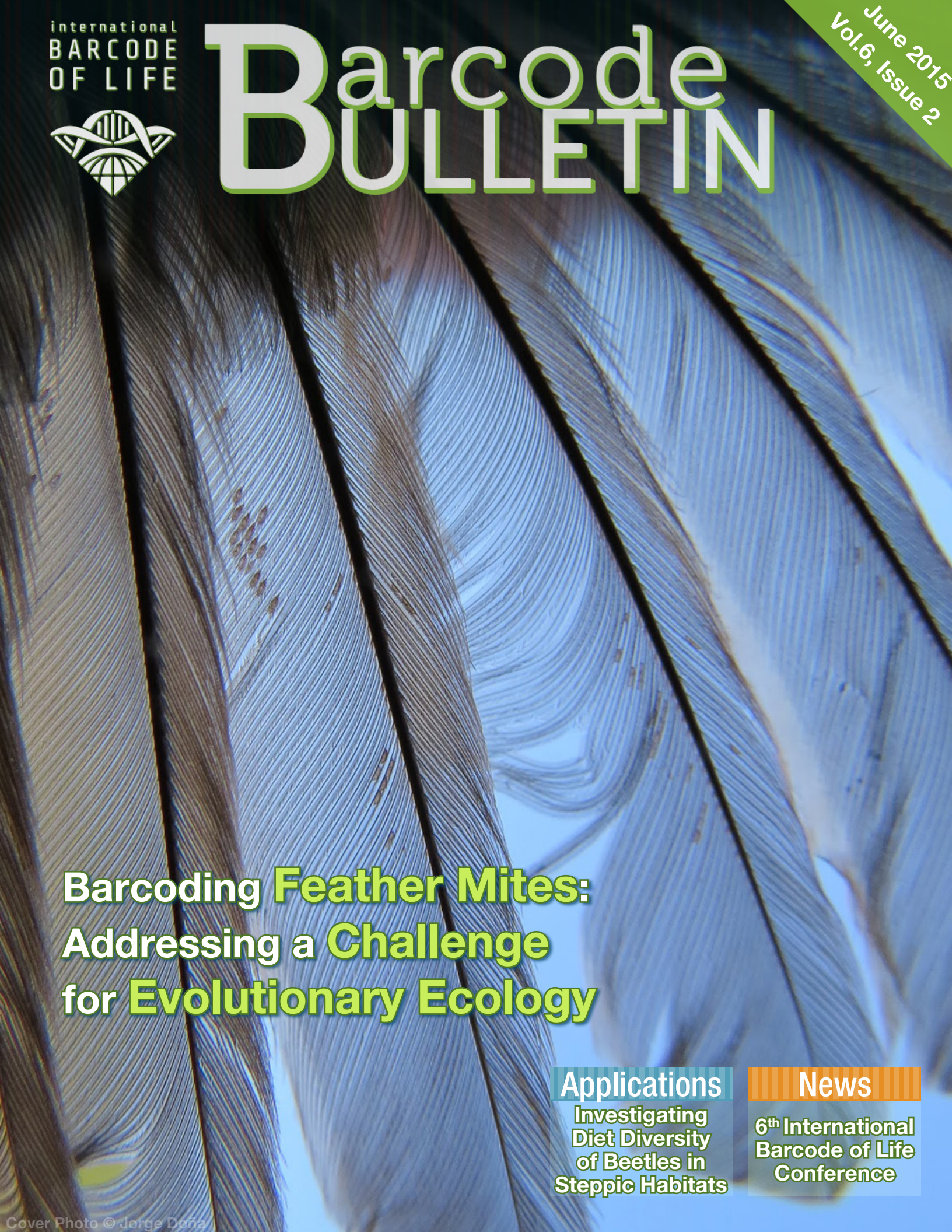

Barcoding Feather Mites: Addressing a Challenge for Evolutionary Ecology

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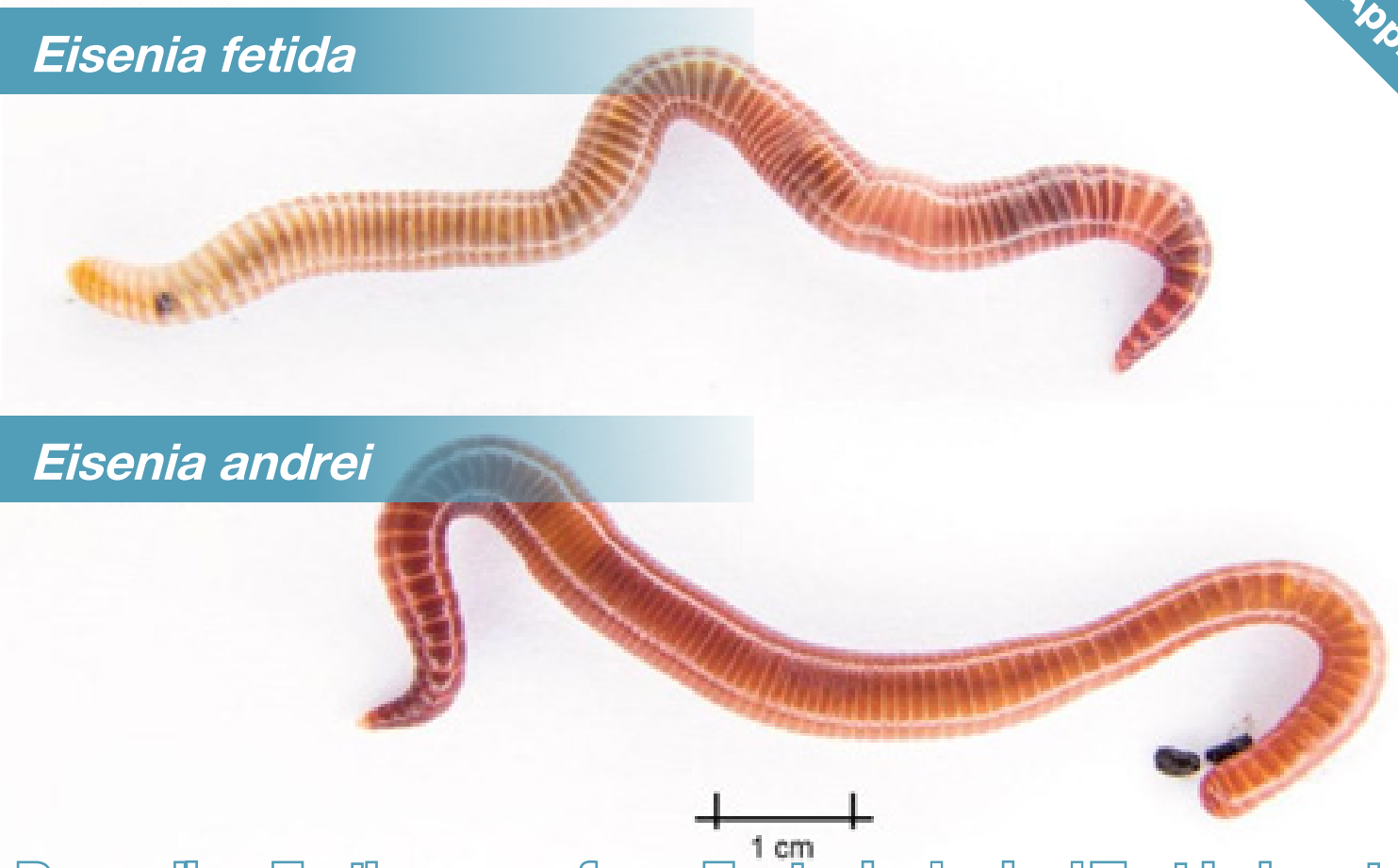

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Barcoding Earthworms from Ecotoxicological Test Laboratories

Written by: Jörg Römbke (ECT Oekotoxikologie GmbH, Germany), Manuel Aira, Thierry Backeljau, Karin Breugelmans, Jorge Domínguez, Elisabeth Funke, Nadin Graf, Mehrdad Hajibabaei, Marcos Pérez-Losada, Pablo G. Porto, Rüdiger M. Schmelz, Joaquín Vierna, Antón Vizcaíno and Markus Pfenninger

Plenty of evidence indicates that the earthworms *Eisenia fetida* (Savigny, 1826) and *E. andrei* Bouché, 1972 (Lumbricidae) can be distinguished by morphological, physiological and molecular traits. However, the morphological differences alone do not allow correct identification of these taxa. This may be a serious problem for the reliability of the standard ecotoxicological tests for which these worms are used.

DNA barcoding is a reliable and practical method for identifying Eisenia species.

In order to assess the practicability and reliability of DNA barcoding, an international ring test was organized by the "*Eisenia* Barcoding Initiative (EBI)", a group of scientists from four public institutions and two contract laboratories. Coded samples of *Eisenia fetida*, *E. andrei*, and *Eisenia* sp. were provided by 28 ecotoxicological laboratories from 15 countries on four continents. Five laboratories in Belgium, Canada, Germany, and Spain identified the specimens through DNA barcoding. All steps of the sample preparation were described by Standard Operating Procedures (SOP).

The COI sequences (581 bp) obtained were used to construct a neighbor-joining tree based on the uncorrected pairwise p-distance (Figure 1). This analysis revealed three distinct haplotype clusters: one including only *E. andrei* sequences (mean within-group p-distance 0.026 ± 0.002) and two with only *E. fetida* sequences, referred to as *E. fetida* 1 and *E. fetida* 2. Each of the latter two in fact represented one single haplotype.

Only 17 out of 28 test laboratories were correct in their taxonomic assignment.

The existence of a cryptic species pair within E. fetida is a plausible hypothesis.

The mean p-distance between *E. fetida* 1 and *E. fetida* 2 was 0.112, whereas the mean p-distances between these two taxa and *E. andrei* were 0.142 and 0.143, respectively. Such COI divergence levels are usually indicative of species level differentiation. Hence, it is hypothesized that *E. fetida* 1 and *E. fetida* 2 refer to different cryptic species.

As the attribution of the individual worms to these three clusters was completely consistent among the five DNA barcoding laboratories, the applicability of DNA barcoding for the identification of these ecotoxicological test species is proven. Remarkably, specimens of the molecular *E. fetida* clusters were always identified morphologically as *E. fetida*. However, this was not true the other way round, i.e. some specimens of the molecular *E. andrei* cluster were identified morphologically as *E. fetida*.

Earthworms used for ecotoxicological tests should regularly be (re-)identified.

The results of this ring test were presented to standardization organizations (OECD, ISO) in order to improve the standardization and thus the quality of ecotoxicological routine testing by using DNA barcoding.

For more information about the results discussed in this article and for full affiliations of the authors, see DOI: 10.1016/j.apsoil.2015.02.010

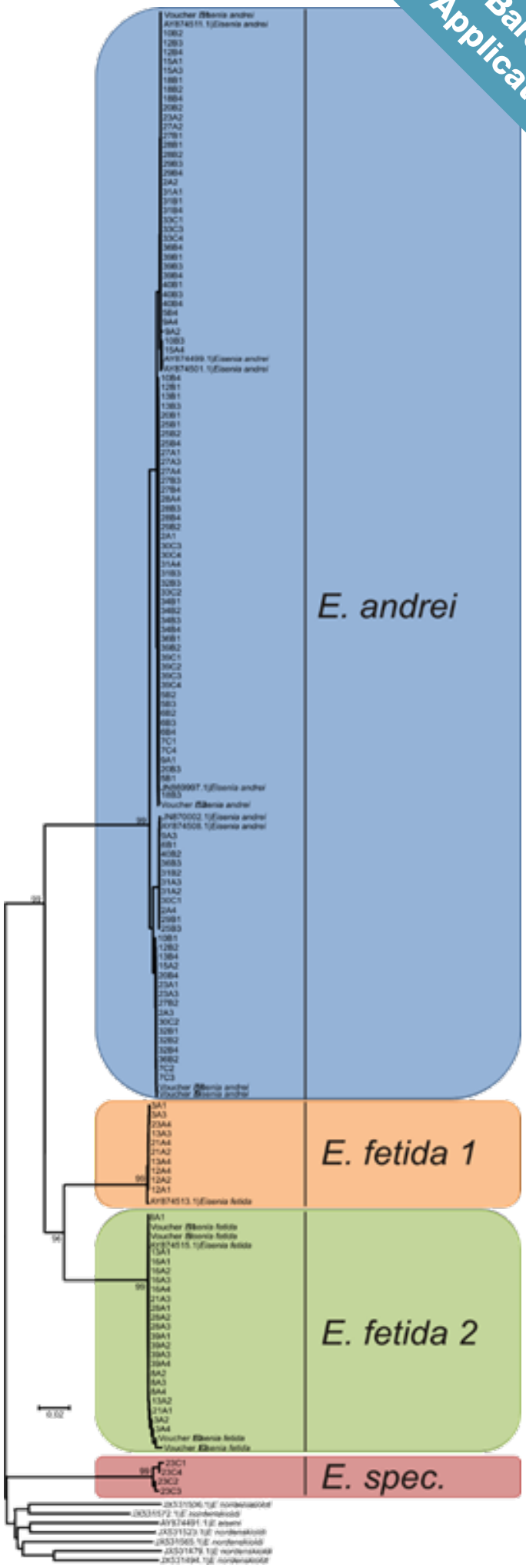

Figure 1 on right: Neighbor-joining tree of 154 test sequences together with morphologically identified voucher specimens and sequences from DDBJ/EMBL/GenBank.