MOLECULAR IDENTIFICATION OF A HITCHHIKING FROG
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The introduction and expansion of non-native species into an ecosystem can be detrimental and result in the decline of native species abundance and the possible extinction of native species (Dorcas and others 2012). Most commonly, the mode of introduction is unintentional and human driven. Many intentional introductions historically have been motivated by individuals or groups who believe that the newly introduced species will be in some way beneficial to humans in its new location (Pimentel and others 2005). Conversely, unintentional introductions are most often a byproduct of human movements, and are thus unbound to human motivations. Introduced species can have negative impacts on native populations, including population declines through niche displacement, interspecific competition for food and habitat, direct predation, and competitive exclusion (Suarez and others 2005).

In this study, we used forensic molecular techniques to identify a stowaway frog that was shipped to Kirkland, Washington, in an Amazon.com package. The frog was deceased and of unknown origin, despite our best attempts to locate the original shipping location from Amazon and its subsidiary shipping companies. It was also severely desiccated, which made it difficult to identify to species using morphological characteristics alone (Fig. 1).

The specimen arrived in a shipment of stereo equipment from Amazon.com on 19 February 2011. The specimen (Fig. 1) has been deposited at the Burke Museum of Natural History and Culture (UWBM 3483). Tissue samples from the frog were removed from the 2nd tarsal of the left foot, in addition to 1 mm² of skin from the lower abdomen. We extracted DNA using a Qiagen DNEasy Blood and Tissue kit, using the spin-column animal-tissue protocol. DNA samples were diluted to a concentration of 10ng/µl prior to PCR amplification.

We targeted the mitochondrial DNA 16S rRNA gene (16S), because this gene has been widely used in amphibian systematics and is currently the most useful marker for molecular taxonomic identification and DNA barcoding in frogs (Vences and others 2005). We amplified and sequenced a 550-bp region of the 16S gene using standard amphibian primers 16SA-L and 16SB-H (Vences and others 2005). The 20 µl PCR reactions included 13 µl of dH₂O, 0.2 µl of Taq polymerase (1 unit), 0.2 µl of 25 mM MgCl₂, 2.0 µl of 10× PCR buffer, 0.5 µl of each 16S primer (20 µM), 0.8 µl of 10 mM dNTP, and 1.0 µl of DNA. The PCR amplification program consisted of an initial denaturation step at 2 min at 94°C. This was followed by 29 cycles of denaturation (30 s at 94°C), annealing (30 s at 48°C) and extension (30 s at 72°C), and a final extension of 5 min at 72°C. PCR products were visualized using a 1% agarose gel stained with EtBr. A sample of Lithobates pipiens (Northern Leopard Frog) was used as a positive control. The PCR products were purified using ExoSAP-IT (USB). We sequenced using dye-labeled