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Introduction of *Eleutherodactylus planirostris* (Amphibia, Anura, Eleutherodactylidae) to Hong Kong

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Abstract. An unidentified small frog species was first encountered in Hong Kong Special Administration Region (SAR), China, in 2000, where the local amphibian diversity is well-studied. We herein identified this unknown frog as *Eleutherodactylus planirostris* (greenhouse frog) using DNA barcoding. We found that its distribution in Hong Kong is widespread (>18 localities), and breeding has been observed in multiple occasions. The populations in at least four localities persisted for over seven years. We discuss its potential negative impacts to terrestrial ecosystems in Hong Kong, with particular concern of its potential competition with the endemic *Liuixalus romeri*. We call for studies to investigate the impacts of the introduced *E. planirostris* on the local ecosystem. Screening for *E. planirostris* in exported plants from Hong Kong should be carried out.

Keywords. Amphibians, biological invasions, greenhouse frog, *Liuixalus romeri.*

The amphibian diversity of Hong Kong, is well studied (Lau, 1999). Owing to the subtropical climate, altitudinal range (highest peak at 957m) and variable terrain it contains a rich mixture of habitats. As a result the highly urbanized Hong Kong is home to a surprisingly high species richness of amphibians [23 species of frogs and 1 species of salamander (Chan et al., 2005)]. In 2000, a morphologically distinct frog not assigned to any of the known native species was captured by the first author in a container yard at Hung Shui Kiu in Hong Kong (Fig 1, Table 1). Subsequently, additional frogs with similar morphology have been found at 18 localities between 2002 and 2015. In Asia-Pacific region, *E. planirostris* has been recorded in Guam (Christy et al., 2007a) and the Philippines (Olson et al., 2014; Sy et al., 2015; Sy and Saigo, 2015); to our knowledge, this is the first reported case of *E. planirostris* introduction and establishment in continental Asia.

This study was conducted in Hong Kong Special Administrative Region, China. Distribution data of this frog species was compiled from region-wide opportunistic surveys between 2000 and 2015. Geographic locations of where this species occurred were recorded using a handheld GPS unit. Two adult specimens were collected from Wu Kau Tang in 2013 for molecular analysis and were deposited at The Museum of Biology, Sun Yat-sen University (Museum voucher number: SYS a004514 and SYS a004515). Genomic DNA from the two specimens was extracted using a DNA extraction kit (FavorPrep Tissue Genomic DNA Extraction Mini Kit, Favorgen Biotech Corporation, Ping-Tung, Taiwan) following manufacturer's protocol and a 562-bp fragment of ribosomal 16S gene was

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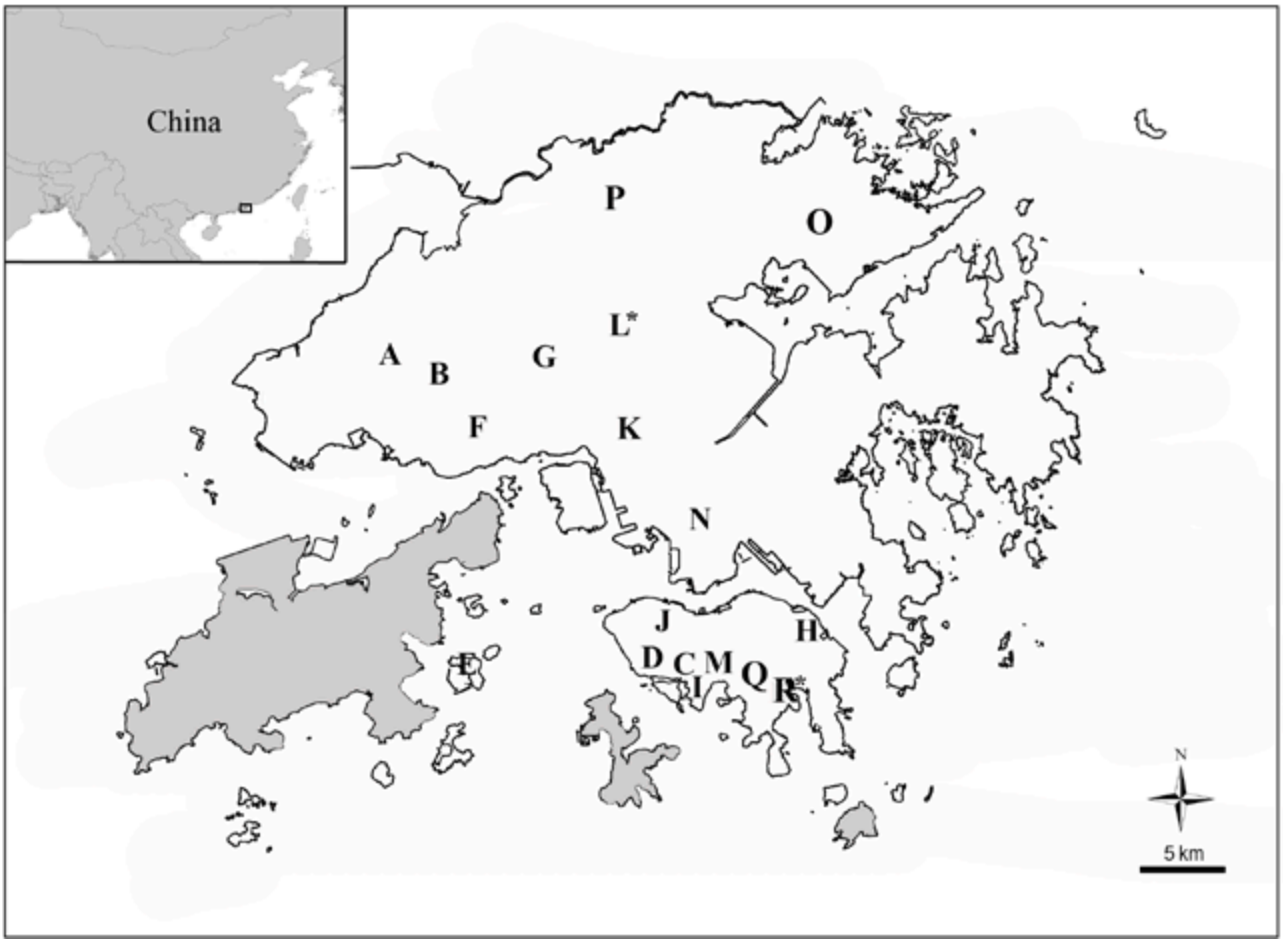

Fig. 1. Locations where *Eleutherodactylus planirostris* were found in Hong Kong. Letters identify the localities, as presented in Table 1. The grey area indicates natural range of *Liuixalus romeri*. Asterisks indicates distribution localities of *E. planirostris* that overlap with translocation sites of *L. romeri*.

amplified using Polymerase Chain Reaction. The resulting products were sequenced on an ABI Prism 3730 automated DNA sequencer and the results were submitted to the program BLAST to identify similar species from the GenBank database and program MOLE-BLAST to produce phylogenetic tree. Genetic sequences have been deposited in GenBank (GenBank accession number KM252679 and KM252680). A study has since been carried out to confirm the identity of this unknown species and to document its distribution in Hong Kong. The unknown frog has been identified as *Eleutherodactylus planirostris* (greenhouse frog), a direct-developing Eleutherodactylid native to a number of Caribbean islands (Bahamas, Cayman Islands and Cuba) (Dodd, 2013). It has been accidentally introduced to many countries throughout the New World (Olson et al., 2012; Andrew et al., 2011; Heinicke et al., 2011), and Asia-Pacific Islands including Hawaii (Kraus, 1999), Guam (Christy et al., 2007a) and the Philippines (Olson et al,. 2014; Sy et al., 2015; Sy and Saigo, 2015), most likely via the live plant trade.

The sequences of the frogs collected from Wu Kau Tang had 100% max identity with the sequences of *E. planirostris* collected at Naples, Collier, Florida (GenBank accession number: DQ283107; Fig. 2) (Frost et al., 2004).

Four sites (Aberdeen, Pokfulam Country Park, Tai Lam Country Park and Tai Tong) were revisited between 2014 and 2015 and robust *E. planirostris* populations were observed at all four sites.

E. planirostris appear to be habitat generalist in Hong Kong, occupying a diversity of habitats including secondary forests, shrubland, agricultural fields, near fishponds, urban parks and near human settlements such as village houses and container yards. We found 22 eggs on wet leaf litter in Aberdeen in May 2006 and the eggs hatched after two weeks in captivity. Egg masses were also found in Pokfulam Country Park and Tai Tong in July 2007.

Table 1. Distribution of *Eleutherodactylus planirostris* and the respective year of discovery in Hong Kong.

Site label	Locations	GPS coordinates	Year of discovery
A	Hung Shui Kiu	22°25.62'N, 113°59.59'E	2000
B	Tai Tong	22°25.25'N, 114°1.33'E	2002
C	Aberdeen Reservoir	22°15.34'N, 114°9.59'E	2006
D	Pokfulam Country Park	22°16.03'N, 114°8.34'E	2007
E	Hei Ling Chau	22°15.41'N, 114°1.90'E	2007
F	Shek Kong	22°25.46'N, 114°6.38'E	2007
G	Tai Lam Country Park	22°24.15'N. 114°2.19'E	2007
H	Chai Wan	22°15.53'N, 114°13.65'E	2008
I	Tai Shue Wan	22°14.19'N, 114°9.96'E	2010
J	University of Hong Kong	22°16.88'N, 114°8.24'E	2010
K	Shing Mun Country Park	22°22.83'N, 114°8.56'E	2011
L	Kadoorie Farm and Botanic Garden	22°25.98'N, 114°7.06'E	2011
M	Nam Fung Road	22°15.26'N, 114°10.68'E	2012
N	Shek Kip Mei	22°19.88'N, 114°10.09'E	2012
O	Wu Kau Tang	22 30.33'N, 114 14.63'E	2012
P	Long Valley	22°30.64'N, 114°6.768'E	2012
Q	Wong Nai Chung Reservoir	22°15.44'N, 114°11.75'E	2013
R	Tai Tam Country Park	22°15.54'N, 114°12.11'E	2015

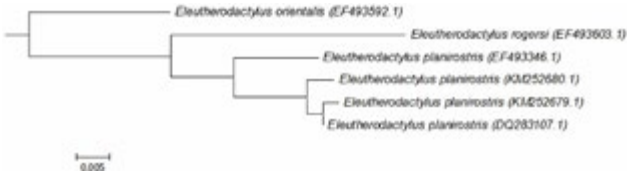

Fig. 2. Phylogenetic tree derived from partial fragments of 16S rRNA genes using fast minimum evolution method produced by program Mole-Blast.

Several individuals kept in captivity from 2009 to 2011 readily ate small crickets, spiders, termites and fruit flies. They produced egg clutches containing 11-17 eggs.

E. planirostris feed on invertebrates including insects, spiders and snails (Global Invasive Species Database, 2010). In Hong Kong, *E. planirostris* is sympatric with eight species of anurans (*Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Hoplobatrachus rugulosus*, *Kaloula pulchra*, *Liuixalus romeri*, *Megophrys brachykolos*, *Odorrana chloronota* and *Polypedates megacephalus)*. However, little is known about the diet of *E. planirostris* in the wild in Hong Kong and the population status of terrestrial invertebrates in Hong Kong, thus its impacts on native invertebrate populations remain unknown.

In the originally amphibian-free Hawaii, introduced *E. planirostris* can exist in high density, where it competes for food with other insectivores (Olson et al., 2012; Beard et al., 2009; Beard and Pitt, 2005) including birds (Global Invasive Species Database 2010). The congeneric *Eleutherodactylus coqui* is listed as one of the world's worst invasive alien species by IUCN (Lowe et al., 2000). On the ecosystem level, the introduction of *E. coqui* can alter ecosystem functioning by distorting nutrient cycling in Hawaii (Sin et al., 2008), however the impacts caused by *E. coqui* can be outweighed by that caused by invasive plants (Tuttle et al., 2009). The impact of *E. planirostris*'s introduction on the endemic *L. romeri* is of potential concern. As adult *E. planirostris* are smaller than most of the frog species in Hong Kong, we speculate that resource competition will be most intense with the similar-sized, small bodied *L. romeri* (Lau and Zhao, 2004). Competition between *E. planirostris* and *L. romeri* might be significant because (1) their sizes are similar and *E. planirostris* consume arthropods (Olson et al., 2012) that overlap broadly with the natural preys of *L. romeri* (Lau, 1998) which indicates a potentially high trophic similarity and (2) we observed *E. planirostris* dwell on the forest floors in secondary forests and forest edges, where *L. romeri* inhabit. We found *E. planirostris* at Tai Lam Country Park and Kadoorie Farm and Botanic Garden, where populations of *L. romeri* were translocated which was a mitigation measures of the construction of the Chek Lap Kok airport in 1990s (Lau and Banks, 2008). *E. planirostris* has yet to be found in the natural range of *L. romeri*, including Chek Lap Kok, Lamma Island, Lantau Island and Po Toi Island (Lau, 1999; Fig. 1). Monitoring of *E. planirostris* should be carried out in the *L. romeri* range and further studies are urgently needed to understand the

impacts of the introduced *E. planirostris* to the *L. romeri*.

Our results demonstrate that *E. planirostris* have established in Hong Kong. The populations at several sites, i.e., Aberdeen, Pokfulam Country Park, Tai Lam Country Park, Tai Tong, persisted for at least seven years. The reproduction of *E. planirostris* is successful in Hong Kong because (1) it is a direct-developing species (i.e. no aquatic larval stage) (Goin, 1947), and (2) the suitable subtropical climate of Hong Kong [annual mean temperature = 22.8ºC; mean annual precipitation = 2214 mm (Dudgeon and Corlett, 2004)] that matches the climate niche of the species (Rödder and Lötters, 2010). Numerous control methods have been carried out to control *E. coqui* populations in Hawaii, and are expected to have similar effectiveness on *E. planirostris* (Olson et al., 2012). It is noteworthy that control methods for *E. coqui* are only effective in eradicating small and isolated populations (Beard and Pitt, 2005). If *E. planirostris* is found to have significant ecological impacts in Hong Kong, prompt control and eradication methods should be executed before the populations become too large.

It is likely that *E. planirostris* was introduced accidentally to Hong Kong via the live plant trade, which was similar to the introduction to Hawaii and Guam (Kraus et al., 1999; Christy et al., 2007b). Large volume of live plants was imported to Hong Kong from continental United States of America in early 2000s when *E. planirostris* was first detected in Hong Kong (Census and Statistics Department, Hong Kong SAR, 2001). This was supported by one frog being found in a potted *Tillandsia cyanea* plant bought from the flower market in Mong Kok in urban Kowloon in 2011 (Louis Fung, per. comm.) and another frog found in an apartment on Ap Lei Chau in 2015 that was likely to arrive together with an indoor plant. In addition, we made observations of high densities of frogs (> 30 in 200 m^2) near newly renovated slopes where nursery plants were planted on. Due to the extensive trade of live plants in the region, it seems it is a matter of time that *E. planirostris* will spread to other places that import plants from Hong Kong. In 2015, Hong Kong exported or re-exported over 100,000 kg of plants or parts of plants to tropical or subtropical countries/cities, including Australia, China, Macau, Malaysia, Singapore, Thailand, Taiwan and Vietnam, where the climate may be favorable to the colonization of *E. planirostris* (Census and Statistics Department, Hong Kong SAR, 2015). Screening for *E. planirostris*, including adults and eggs, in exported or re-exported plants from Hong Kong should be carried out.

The small size and variations in coloration of *E. planirostris* as well as morphological similarity with certain Asian species (e.g., *Liuixalus* sp. and *Philautus* sp.) prevented identification before molecular approach was applied to confirm its identity (Armstrong and Ball, 2005). This delayed proper measures to be taken to control this introduced species. Prompt molecular analysis to identify suspicious alien species should be encouraged. We also hope this paper will help arouse the attention on this introduced frog in Asia.

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