New localities for *Tylototriton uyenoi*, *T. panhai* and *T. anguliceps* in Thailand with remarks on the southernmost distribution of the genus

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INTRODUCTION

he genus Tylototriton inhabits tropical and subtropical, moist broad-leaf forests close to waterbodies at moderate to high elevations between 1,250 and 1,900 m above sea level (m a.s.l.) in Thailand (Taylor, 1962; Wongratana, 1984; Nabhitabhata & Chan-ard, 2005; Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2016a,b; Dowwiangkan et al., 2018). Three species were reported in the region, Tylototriton uyenoi, Tylototriton panhai and Tylototriton anguliceps (Nishikawa et al., 2013; Le et al., 2015). These crocodile newts are distributed throughout northern Thailand but their occurrence and ecological requirements are poorly known (Hernandez, 2015, 2016a,b; Hernandez et al., 2018). Tylototriton uyenoi occurs from the Dawana and the Daen Lao Hills and from the Phi Pan Nam to the Inthanon Range (Gerlach, 2012; Michaels, 2015; Hernandez, 2015, 2016a,b, 2017; Dowwiangkan et al., 2018). The Daen Lao Hills are a mountain network that extends to the southern Shan states and Karen Hills (Kayah and Kayin States bordering Mae Hong Son) in Myanmar. This mountain system is connected to the north-western mountains of Thailand where T. uyenoi can also occur (Hernandez, 2017). Tylototriton panhai inhabits mixed deciduous, dry dipterocarp, dry evergreen, and hill evergreen forests at elevations between 1,285 to 1,688 m a.s.l (Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2016a,b, 2017). Its presence is known from Phitsanulok, Uttaradit, Phetchabun and Loei Provinces in north-eastern Thailand, and east into Sainyabuli Province, Botene District, Laos (Wongratana, 1984; Nabhitabhata & Chan-ard, 2005; Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2015, 2016a,b; Phimmachak et al., 2015). Tylototriton anguliceps inhabits evergreen forests at elevations of 1,300-1,800 m a.s.l in north-western Vietnam, northern Laos and north-eastern Thailand (Le et al., 2015; Phimmachak et al., 2015). Here we report four new records for these Tylototriton species in northern and north-eastern Thailand and we discuss their biogeographical preferences (Fig. 1).

MATERIALS AND METHODS

We conducted field work over a three-year period 2014–2016 and 2018 respectively, during the monsoon season (April to September) in north-western, northern and north-eastern Thailand.

Observations of the vegetation and habitat of salamanders were made during field trips both on sunny and on rainy days from approximately 08:00 h to 23:30 h. We surveyed almost all types of habitat which included permanent and temporary streams but also ponds (including artificial reservoirs and irrigation canals), and surrounding terrestrial habitats, stumps, stones and litter. For identification purposes, we photographed using a digital camera (Sony Nex-5; Sony Ltd., Japan) each taxon observed, including plant and tree species. Coordinates, geographic and elevational data were collected in situ using a Global Positioning System (Garmin Montana 680; Garmin Ltd., Olahe, KS, USA) and located on maps. Water pH and temperature was recorded in situ using a Expresstech @ LCD PH Medidor Digital (Expresstech; Kingpow Company Limited; Hong-Kong; China). We also assessed the relative positions of the species on environmental gradients using principal component analysis (PCA). This allows us to visualise if these new localities are within the ecological range (95 % confidence ellipses) expected for these species (Hernandez et al., 2018). For this we followed the protocol of Hernandez et al. (2018), including the mean annual temperature (°C), temperature seasonality, annual precipitation (mm), and precipitation seasonality, from the WorldClim database (Hijmans et al., 2005). We also included a descriptor of the topography (index of terrain ruggedness) and the percentage of forest cover (Tuanmu & Jetz, 2014). The variables were normalised before the PCA was implemented using the Paleontological Statistics package (PAST; Hammer, 2015).

RESULTS

We found *T. uyenoi* at Doi Mon Jong, Tak province, northwestern Thailand (17°32'12.18" N, 98°31'47.30" E) 1,597 m

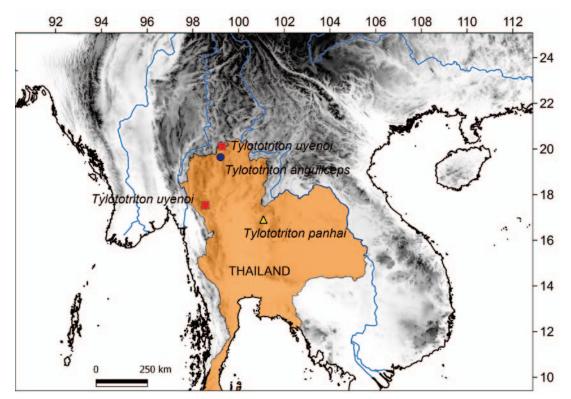


Figure 1. Map of Thailand (shaded orange) and neighbouring countries, showing the four new locations described in this study: Blue circle, *T. anguliceps*; Red squares, *T. uyenoi*; Yellow triangle, *T. panhai*

a.s.l. One adult male (Fig. 2A) was found near a small pond in a montane evergreen forest. Four eggs were also recorded from the same pond. They were deposited one by one in the submerged vegetation. The finding confirmed that this species lays eggs underwater like other species of the subgenus *Tylototriton* (Raffaëlli, 2013; Hernandez, 2016a,b). We found *T. uyenoi* at Doi Mak Lang, Mae Ai district, Chiang Mai province, northern Thailand (20°6'47" N, 99° 15'37" E) 1,456 m a.s.l. One adult male was observed under a large rotten tree in a hill evergreen forest dominated by *Ostodes paniculata, Canarium bengalense* and *Polyspora axillaris* (Fig. 2B). The air temperature was 21.5 °C with a relative humidity of 76 %. This specimen was blackish-to brown in colour and showed robust parotoid glands, being similar to topotypic *T. verrucosus*. It measured a total length of 168 mm (TL).

Furthermore, on 12 July 2014, three adults of T. panhai were found at Phu Thap Boek, Lom Kao district, Phetchabun province, north-eastern Thailand (16°54'23"N, 101°5'14"E) 1,663 m a.s.l. These individuals were observed in a small stream. One adult male measured 132 mm (TL) showing the typical traits of the "type III" (Fig. 3A; see Hernandez 2016a,b, 2017): it showed a dark reddish brown colouration with 11-13 small reddish dorsolateral glandular warts (sometimes indistinct in adult specimens). These warts were placed on the dorsum, laterally, very close to each other. The shape of the head and the cephalic edges were also more angular than in other phenotypes. The vegetation around the stream included trees and giant perennial grasses of the genus Musa. The air temperature was 26.4 °C, water temperature 20.2 °C with a relative humidity of about 80.9 %. The water was slightly acidic (pH 6.62). We recorded T. anguliceps at Si Dong Yen, Chai Prakan district, Chiang Mai province, northern Thailand during August in the same year 2014 (19°38'31.8"N 99°12'44.4"E) at 1,260 m a.s.l. We found one adult male hiding under large rocks in a dry evergreen forest near a slow-flowing stream (Fig. 3B). The air temperature was 22.1 °C with a relative humidity of 73.2 %.



Figure 2. A. Adult male of *T. uyenoi* found at Doi Mon Jong, Tak province, north-western Thailand **B.** Specimen of *T. uyenoi* at Doi Mak Lang ("group II" from Hernandez, 2016a,b) which is more related to *T. verrucosus* topotypic from Husa, Longchuan county, Yunnan province, China



Figure 3. A. One male individual of *T. panhai* found in 2014 at Phu Thap Boek, Phetchabun province, north-eastern Thailand **B**. Specimen of *T. anguliceps* from Doi Lahnga range which is located 100 km south from our new locality in Si Dong Yen, Chiang Mai province, northern Thailand

DISCUSSION

These important new records confirm that crocodile newts of the genus Tylototriton inhabit mountainous moist evergreen forests at medium to high altitudes (Hernandez, 2016a,b, 2017; Hernandez et al., 2018). In the PCA, the first axis explained 54.0 % of the variance and the second axis the 23.3 %. In the first axis the localities were segregated in a gradient of climate seasonality, precipitation and temperatures (factor loadings: temperature seasonality = 0.54, precipitation seasonality = 0.51, mean annual precipitation = 0.53 and mean temperature = -0.37). In the second axis, the localities were segregated in a topographic and forest cover gradient (factor loadings: terrain ruggedness = 0.48, forest cover = 0.70), negatively associated with temperatures (mean temperature = -0.46). The PCA showed that these new sites were within the expected macroecological niche for these species, although in the case of T. uyenoi, these localities occupied two extremes within the environmental gradient (Fig. 4). However, macroecological models could fail to describe the niche of species that are closely linked to densely forested habitats (Scheffers et al., 2014); for this reason future studies should be directed to confirm this preliminary result, based on fine habitat characterisation. Moreover, the northernmost localities found in Thailand are Doi Mak Lang, Chiang Mai province for T. uyenoi (this paper), Phu Soi Dao, Uttaradit province for T. panhai (Hernandez, 2017) and Si Dong Yen for T. anguliceps (this paper). However, phylogenetic studies are needed to confirm the taxonomic status of these new populations. The Doi Mak Lang type differs in colour pattern and external morphology by having large cephalic edges, pronounced parotoid glands, skin finely granular, general ground colour dark brown above, glands on the neck and in the dorsolateral regions lighter brown to reddish orange. Indeed, Tylototriton species show a

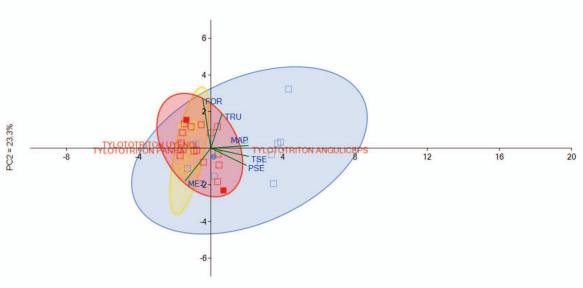




Figure 4. Crocodile newts ecological space, based on PCA. The 95 % confidence ellipses are shown. FOR: Forest cover; MAP: Mean Annual Precipitation; MET: Mean annual temperature; PSE: Precipitation seasonality; TSE: Temperature seasonality: TRU: Terrain ruggedness. Blue, *T. anguliceps*; Red, *T. uyenoi*; Yellow, *T. panhai*. The new records are shown as filled figures, those described in Hernandez et al., 2008 as empty squares.

conservative morphology that makes it difficult to differentiate between some species (Nishikawa et al., 2013; Le et al., 2015; Phimmachak et al., 2015). Genetic and ecological studies can provide new clues to classify these cryptic species (Hernandez et al., 2018).

Concerning T. panhai, we found three main phenotypes located in north-eastern Thailand (see Hernandez, 2016a,b) while for *T. uyenoi*, we found two main phenotypes including: (i) a northern and north-western type (including the type locality: Doi Suthep, Chiang Mai province); (ii) a north-eastern type which is similar to T. verrucosus sensu stricto. However, this latter group would require phylogenetic analyses to determine its true taxonomic affinities (Hernandez, 2015, 2016a,b, 2017). Regarding T. anguliceps we confirmed its presence in the three previously known localities in Thailand: Doi Lahnga, Doi Wiang Pha (Hernandez, 2015, 2016a; Le et al., 2015) and Si Dong Yen (this paper). All of these localities are within the same mountain range (pers. obs.). Furthermore, three other known localities of T. uyenoi are distributed through Tak province along the Dawana hills comprising: Doi Soi Malai, Umphang (Hernandez, 2015, 2016a,b, 2017) and Doi Mon Jong (this paper). These records constitute the southernmost localities for the species but also, for the whole genus Tylototriton extending 200 km southwards of its known distribution (Hernandez, 2017; Hernandez et al., 2018). Moreover, nearby mountains also have suitable habitats and could harbour undiscovered populations. More surveys are needed to improve knowledge about the distribution of these threatened crocodile newts and to establish conservation priorities

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