

The first record of green toads *Bufo* sp. from the island of Ios, Cyclades, Greece, with notes on the distribution and conservation of green frogs *Pelophylax kurtmuelleri* (Gayda, 1940) on Ios and Milos

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Introduction

The Cyclades Archipelago comprises about 20 Greek islands in the Aegean Sea, centred around the capital island of Syros (Gaki-Papanastassiou *et al.*, 2010). Most of these islands, formed by metamorphic and igneous processes (Hejl *et al.*, 2002), are the peaks of previously exposed mountains. The numerous small islands outcrops on the plateau today are the result of a complex geomorphology (Kapsimalis *et al.*, 2009), with the exception of the volcanogenic islands of Milos and Santorini (Gaki-Papanastassiou and Papanastasiou, 2014). Milos hosts a unique herpetofaunal diversity that includes four taxa endemic to the Milos archipelago (Vervust *et al.*, 2013): the viper *Macrovipera schweizeri* (Werner, 1935), the wall lizard *Podarcis milensis* (Bedriaga, 1882), the Balkan green lizard *Lacerta trilineata hansschweizeri* (Müller, 1935), and the grass snake *Natrix natrix schweizeri* (Müller, 1932) (Chondropoulos, 1986, 1989). The

presence of so many endemic reptiles makes the existence of an endemic amphibian expectable (Vervust *et al.*, 2013); however, fresh water is a rare commodity in the Cyclades (Kyriakopoulou-Sklavounou, 2000) and this scarcity shapes the distribution of amphibians there. Amphibians known from the Cyclades include *Bufo bufo* (Linnaeus, 1758), *Hyla arborea* (Linnaeus, 1758), water frogs (genus *Pelophylax*; Broggi, 2000, 2007, 2011; Valakos *et al.*, 2008) and green toads (genus *Bufo*), most of them occurring only on some islands (Lymberakis *et al.*, 2007; Sillero *et al.*, 2014). The taxonomic status of both of the latter genera in the Cyclades remains unclear at the present time (Vervust *et al.*, 2013; Dufresnes *et al.*, 2018, 2019).

The Balkan water frog (*Pelophylax kurtmuelleri* (Gayda, 1940)) is distributed primarily in Greece but extends into southern Albania, with introductions elsewhere (AmphibiaWeb, 2008). Detailed information



Figure 1. Male Green toad *Bufotes* sp. identified at Mylopotas, Ios, Cyclades, Greece (Photo by Stuart Graham).

on the species' life history etc., as distinct to other *Pelophylax* species is notably lacking. Vervust *et al.* (2013) suggested that Balkan water frog on Milos were quite distinct as compared to other *P. kurtmuelleri* populations and these Cycladic island forms should at least be considered “evolutionary significant units” (ESUs).

The green toad (*Bufotes viridis* (Laurenti, 1768)) sensu lato complex of species extends from North Africa, throughout much of Europe, into western and Central Asia (AmphibiaWeb, 2008), and it is considered a

species complex requiring additional taxonomic work (e.g., Dufresnes *et al.*, 2018, 2019). In Greece, green toads are found on the mainland and on most of the islands (Valakos *et al.*, 2008). Dufresnes *et al.* (2018) discovered a new and distinct mitochondrial lineage of green toads endemic to Naxos (central Cyclades), which appears to be the result of Quaternary sea level changes that lead to vicariant isolation. Dufresnes *et al.* (2019) revised taxonomy asserts that most Greek (including mainland) *Bufotes* populations should be considered *B. viridis*, with *B. sitibundus* occurring in the eastern and central Aegean, however this is as yet

Table 1. Amphibian survey results for the islands of Ios and Milos in the Cyclades archipelago, Greece, in 2018 and 2019. Asterisks (*) indicate a reduced accuracy due to site permissions. The octothorpe (#) indicates site is subject to Presidential Decree 229/2012.

Island	Date	Site type	Coordinates	Species present
Ios	07 April 2019	Garden pond, 120 m East of Mylopotas Reservoir Protected Wetland	36.425°N, 25.182°E*	<i>P. k.</i> adults, tadpoles
	07 April 2019	Reservoir#	36.4252°N, 25.1837°E	none
	08 April 2019	stream pools east of Ostria village	36.4256°N, 25.1828°E	none
	08 April 2019	Wetland	36.7258°N, 25.2740°E	<i>P. k.</i> adult (calling)
	08 April 2019	Seasonally flooded area, 1.16 km west from East of Mylopotas Reservoir Protected Wetland	36.7147°N, 25.2955°E	<i>B. v.</i> adults
Milos	02 October 2018	Natura 2000 lake#	36.6875°N, 24.4425°E	none
	13 April 2019			<i>P. k.</i> Adults (calling)
	03 October 2018	Two waterbodies (dry in 2018, wet in 2019)#	36.7057°N, 24.4866°E	none
	09 April 2019			<i>P. k.</i> Adults
	04 October 2018	Drainage culvert (wet in 2018, dry in 2019) 0.12 km east of Zefyria (disused) Brickworks Protected Wetland	36.7055°N, 24.4884°E	<i>P. k.</i> Adults, sub-adults
	09 April 2019			none
	09 April 2019	Drainage culvert under road 0.28 km north-east from former salt extraction of Aliko Protected Wetland	36.42485°N 24.29331°E	<i>P. k.</i> Adult
	10 April 2019	Former mine workings	36.7232°N, 24.5249°E	<i>P. k.</i> (possible splash heard)
	11 April 2019	Seasonally flooded area/culverts#	36.7013°N, 24.4842°E	<i>P. k.</i> Adult (calling)
	12 April 2019	Field pond 0.73 km west from Katsaronas Protected Wetland	36.4357°N, 24.3111°E	none
12 April 2019	Field pond	36.4514°N, 24.3138°E	none	

not widely accepted and requires further investigation.

We therefore refer to green toads encountered during this study as *Bufo* sp. (figure 1). This article provides an update on the distribution of amphibians present on the Cyclades Islands of Ios and Milos, along with suggestions for their conservation.

Methods

Surveys were undertaken on the islands of Ios (07–08 April 2019) and Milos (02–04 October 2018 and 09–13 April 2019) in the Cyclades Archipelago, Greece, with the primary objective of identifying extant localities for *P. kurtmuelleri*. A desktop study was conducted

to identify waterbodies and locations on these two islands. A literature review was also undertaken to identify waterbodies and locations on these two islands where *Pelophylax* sp. had been previously recorded. Field surveys adopted methodologies detailed within Wilkinson (2015). Equipment (dip-nets, boots, sample tray and in contact survey equipment) was appropriately sterilised adopting guidelines detailed in ARG UK (2017). Amphibians were identified utilising Broggi (2000), Valakos *et al.* (2008) AmphibiaWeb (2008) and Dufresnes *et al.* (2018). Upon capture or observation, all locations were recorded utilising a Garmin GPSmap 60CSx (+/- 2-5 m) with images of individuals taken with a Ricoh WG-50 (16MP).

Results

Survey results are presented in Table 1. We encountered *P. kurtmuelleri* at two of five localities on Ios and at six of eight localities on Milos. We made only a single observation of *Bufo* sp. on Ios.

Discussion

This study presents the first published record of the occurrence of green toads on the island of Ios and the first published update on the amphibian fauna of Milos since Broggi (2000). Grey literature suggests the presence of green toads on Ios, however this is the first confirmed published record of green toads on the Island. Wilson (2019) and Bok (2013) made mention of one unspecified locality of *P. kurtmuelleri* on Ios. This study details the location and confirms the presence of

P. kurtmuelleri on Ios. Since Ios lies merely some 12 km to the south of Naxos, the exact identity of the Ios green toads needs further investigation. Further studies are required regarding genetic analysis and species assignment, along with surveys of additional water bodies across the island to establish distribution.

Substantial rains in the Cyclades over winter 2018-19 may have improved the breeding conditions for (and detection of) amphibians there but, despite this, the persistence of both species on these islands is likely to be conservation dependent.

The apparently small populations of amphibians found on Ios and Milos appear to be particularly vulnerable, experiencing extreme isolation within highly fragmented hydrological systems, and possibly extreme weather-driven fluctuations in population size as a result. Anthropogenic factors result in additional threats to these insular amphibians, increasing their risk of extinction. Small island wetlands are specifically protected in Greece through a Presidential Decree (229/2012). Activities should promote the sustainability of wetland habitats and any development within the wetlands that can affect their integrity and hydrology prohibited. Only light development activities are permitted, that aim to enhance the wetlands and inform the public. Our survey results indicated that only two out of the six (private garden pond not included) wetlands are protected under the Presidential Decree.

Ios, Garden Pond. The pond lies within the grounds of a family house, approx. 262m west of a reservoir protected under the Presidential Decree. There is a high risk for the pond to be lost/grounds to be modified through landscaping. Although not known if this is a relic population or introduction, this breeding locality needs to be maintained in its current state to ensure breeding/overwintering sites are not lost. No other Balkan water frog breeding sites on Ios can currently be confirmed. Further surveys are required to adjacent water systems to better understand if Balkan water frogs are using the ditch systems that lead to the reservoir. The results of this survey may indicate that this ditch system is important for the survival of fragmented Balkan water frog population(s) in this location and should be included under the Presidential Decree.

Ios, Port Wetland. The wetland area showed visual signs of petrochemical pollution. Experimental studies have confirmed the toxicity of these pollutants to amphibians (Heatwole & Wilkinson, 2009). The greatest risk in this area seemed to be future developments as it is located in the middle of a residential, touristic and partly industrial area. Destruction of habitat that leads to fragmentation is perhaps the major present cause of decline in amphibians (Lemckert *et al.*, 2012). High numbers of mosquito larvae were recorded throughout the marshland area. Due to the proximity to residential houses, there is a risk of industrial insecticide use as well as draining of the marshland, to control the mosquito population (e.g., Kolimenakis *et al.*, 2019).

The marshland area needs to be protected against drainage operations and any form of development till further population studies are undertaken.

Ios, Mylopotas Beach. Sandwiched between hotel complexes, located 1.15 km west of a reservoir protected under the Presidential Decree. The greatest risk in this area seemed to be future development. Being adjacent to hotel developments, there is a risk of industrial insecticide use and development of the wetland area to control any mosquito population present and increase business opportunities. Amphibians may also be the subject of persecution at touristic locations in Greece, as observed by Kaczmarek & Zurawlew (2019), perhaps because of some perception of nuisance to visitors. This wetland area needs to be protected against any form of development till further population studies are undertaken. An understanding of the population will provide future management objectives to safeguard the population.

Milos, Achivadolimni Lake. This is the largest natural wetland in the Cyclades. It belongs to the regional network of Natura 2000 sites of the European Union and therefore enjoys a degree of protection. The lake is further protected under Presidential Decree; protection of small wetlands found across the Greek islands. Though we cannot confirm breeding of *P. kurtmuelleri* there from our surveys in 2018 and 2019, it seems likely that this remains at least periodically feasible.

Milos, Brickworks Factory. The brickworks factory is surrounded by farmland and included on the list of small wetlands found across the Greek islands protected under the Presidential Decree. Drainage pipes were observed in the water bodies abstracting water for adjacent agricultural irrigation. Water was recorded only after a wet year (2019), immediately following the winter months. It is therefore considered likely that this current practice could have dramatic effects on the breeding success and persistence of the Balkan water frog population, especially in (the more normal) drier years. It is advised that water abstraction is ceased at this location, and that the ponds be managed to provide a permanent or at least more reliably persistent water body. Further population monitoring is required to formulate a sustainable conservation strategy.

Milos, Road Culverts. Both road culverts, east of the brickwork factory and north of the airport wetland, which may represent a key aquatic refuge while other wetlands are dry, are currently managed using heavy machinery to prevent flooding. This could have a detrimental impact on the survival of the species in this culvert network. We recommend that clearance operations be timed to avoid key breeding and overwintering periods and carried out using light machinery only. In addition, the culverts should be sectionally cleared over a four-year rotational basis to avoid ‘total’ habitat destruction in any one season.

Milos, Katsaronas Mine Workings. We infer the

presence of *P. kurtmuelleri* here only from a distinct “splash” typical of a Balkan water frog diving into the flooded mine workings. However, this waterbody is located 440m north-west to a historically known site, ca. 720 m to the west (36.7222°N, 24.5306°E), which is a pond formed at the bottom of another former open-cut mining site, protected under the Presidential Decree. No Balkan water frogs were heard calling during a repeat visit under favourable survey conditions on the same night.

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