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## New records of Natterjack toad (*Epidalea calamita*, Laurenti 1768) natural breeding sites in Ireland

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The Natterjack toad (*Epidalea calamita*) has a wide distribution throughout Europe, ranging from the Iberian Peninsula to the Baltic coast with several isolated populations in Great Britain and Ireland (Gasc et al., 1997). Despite its widespread distribution, the conservation status for this species has been assessed as ‘unfavourable’ throughout most European populations (European Topic Centre, 2012). In Ireland, the Natterjack toad is at the extreme western edge of its range and is regionally IUCN Red Listed as ‘Endangered’ (King et al., 2011). It is highly range restricted in Ireland confined to the south-west of the country in County Kerry and one small introduced population to the south-east of the country in County Wexford. The latest conservation assessment suggests that the population is declining (Reyne et al., 2019) mostly likely due to the degradation of suitable breeding sites (Beebee, 2002). Ireland lost over half its farmland ponds during the 20<sup>th</sup> century associated with agricultural intensification and large-scale land drainage schemes that destroyed amphibian breeding habitat (Reid et al., 2014). Natterjack toads are presently restricted to seven discreet sites representing metapopulations (named: Magherees, Inch, Rosscullen, Dooks, Yganavan, Glenbeigh and Caherdaniel) (Beebee, 2002).

The Natterjack toad is listed under Annex IV of the EU Habitat and Species Directive (92/43/EEC) with EU member states required under Article 17 to report regularly to the European Commission on species’

population size and trend. At intervals of roughly 6 years, Ireland’s National Parks & Wildlife Service (NPWS) commissions monitoring and surveillance of all known breeding sites. Field surveys occur over 2-3 consecutive years where spawn is recorded every 2 weeks from April to July, to coincide with the breeding season, thereby enabling estimation of the breeding population size (Bécart et al., 2007; Sweeney et al., 2013; Reyne et al., 2019). As part of this program, we conducted extensive field searches for new natural breeding sites from 2016 to 2018 in order to update the species known range in Ireland. Surveys were conducted in County Kerry including the known species occurrence range as well as suitable areas (sand dunes, coastal grasslands and marshes) outside the distribution range. All newly discovered breeding sites were included in the annual survey and visited every 2 weeks after the initial discovery. The perimeter of each potentially suitable water body was surveyed for presence of egg strings by walking slowly along the shore and conducting zigzag transects across shallow water. Sweep netting was used to determine presence of tadpoles. We collected tissue samples from each site where Natterjack toad eggs and tadpoles were detected in order to confirm the species. Samples were stored in 100% ethanol until extraction, which was carried out following a high salt protocol (Miller et al., 1988). A 710 base pair fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified using LCO1490 and HCO2198 primers (Folmer et al., 1994). The polymerase chain reaction and cycling program followed the original protocol (Folmer et al., 1994) but the annealing temperature was increased to 46 °C to reduce nonspecific amplifications. All PCR products occurred at the correct fragment size and were sent to Eurofins Genomics Ltd. for Sanger sequencing. Sequence similarity searches were performed in GenBank BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST>) and BOLD International System (<http://www.boldsystems.org>).

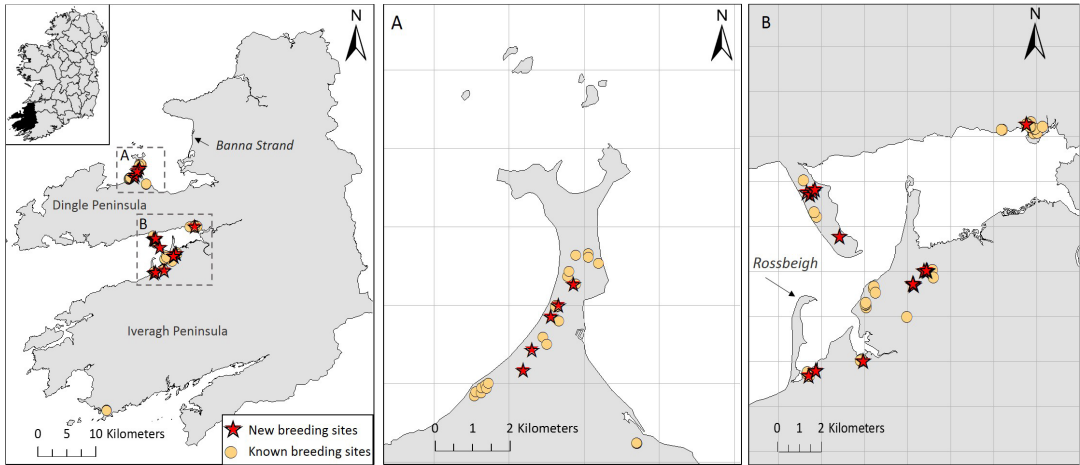
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**Figure 1.** Map of the known and newly discovered Natterjack toad breeding sites in Ireland (insert highlights County Kerry with a 2 km grid). A) Magharees sand dune system. B) Castlemaine Harbour.

**Table 1.** List of newly discovered breeding sites during the 2016–2018 Natterjack toad survey along with their coordinates, date of discovery and type of water body.

Area	Latitude	Longitude	Date	Water body type
Glenbeigh	52.0585	-9.9634	12 April 2016	Temporary puddle
	52.0605	-9.9588	4 June 2018	Temporary puddle
Inch	52.1315	-9.9680	27 May 2016	Temporary pond formed in dune slacks
	52.1306	-9.9659	27 May 2016	Temporary pond formed in dune slacks
	52.1318	-9.9639	27 May 2016	Temporary pond formed in dune slacks
	52.1329	-9.9624	27 May 2016	Temporary pond formed in dune slacks
	52.1142	-9.9449	7 May 2016	Temporary pond formed in dune slacks
Iveragh Quarry	52.9631	-9.9667	5 April 2016	Temporary puddle
Magharees	52.2635	-10.0463	14 April 2016	Lake
	52.2836	-10.0276	8 May 2018	Temporary pond formed in dune slacks
	52.2682	-10.0430	25 April 2016	Temporary pond formed in dune slacks
	52.2786	-10.0332	9 June 2016	Temporary pond formed in dune slacks
	52.2760	-10.0360	25 April 2016	Temporary pond formed in dune slacks
Rosscullen Island	52.1611	-9.8201	25 May 2017	Drainage ditch
Yganavan Lake	52.0962	-9.8941	6 April 2016	Lake
	52.0957	-9.8939	27 April 2016	Lake
	52.1015	-9.8849	27 April 2016	Lake
	52.1014	-9.8871	27 April 2016	Lake
	52.1017	-9.8858	24 May 2016	Puddles formed in wheel tracks
	52.0962	-9.8944	5 May 2016	Lake

All samples were successfully amplified and confirmed to be Natterjack toad. The sequence data have been deposited in GenBank with accession numbers MT372442 – MT372461 (Benson et al., 2017). In total, 20 new natural breeding sites were discovered during the 2016-18 field survey (Table 1, Fig. 1), expanding the known recorded range of the species (at a 2km grid cell resolution) by +19% (with an additional 3 cells occupied) since the last survey during 2011-12 (Sweeney et al., 2013). Half of the new locations were recorded from coastal sand dunes systems. Inch sand dunes (52.2806°N, -10.0299°E) has been historically recognised as important breeding area (Beebee, 2002), however prior to the survey only three breeding sites were known. The discovery of five new sites with high numbers of egg strings (232 in 2018) and tadpoles (>10,000) highlights the ongoing importance of this location in an Irish context. We extensively searched two other sand dune systems in Co Kerry: Banna strand (52.3375°N, -9.8342°E) and Rossbeigh (52.0682°N, -9.9716°E) but no evidence of breeding was found, probably due to high water salinity (>20ppt) recorded at ponds within the dune slacks. Seven of the new locations were found along the shores of two large lakes: Yganavan Lake (52.0954°N, -9.8891°E) and Lough Gill (52.2601°N, -10.0450°E), two unusual breeding sites for the species which typically uses shallow ephemeral ponds, avoiding permanent waterbodies where tadpoles may suffer from increased predation and competition (Griffiths et al., 1991; Stevens and Bagnette, 2008). All lake sites were in small shallow (therefore warm) bays sheltered from wave activity avoiding deep cold water. Breeding activity was also recorded at small temporary puddles formed after heavy rains, where egg string and tadpole survival was likely to be low due to desiccation. A paucity of potentially suitable farmland ponds at the landscape-scale may be a reason for toads selecting habitats that might be otherwise perceived as unsuitable. Continued monitoring of all known breeding locations (including those new locations reported here) will be crucial in determining population trajectories. Maintaining the suitability of breeding sites (preventing ecological succession and controlling the impacts of agricultural intensification) will be necessary to stop further declines.

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