Acta Chiropterologica, 12(2): 457–461, 2010
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS
doi: 10.3161/150811010X538025

The status of the cryptic bat species, *Myotis mystacinus* and *Myotis brandtii* in Ireland

EMMA S. M. BOSTON[1], DANIEL J. BUCKLEY[1], MICHAËL BEKAERT[1], YANN GAGER[1], MATHIEU G. LUNDY[2], DAVID D. SCOTT[2], PAULO A. PRODÖHL[2], W. IAN MONTGOMERY[2], FERDIA MARNELL[3], and EMMA C. TEELING[1, 4]

[1]*Centre for Irish Bat Research, University College Dublin, School of Biology and Environmental Science, Belfield, Dublin 4, Ireland*
[2]*Centre for Irish Bat Research, Queen's University Belfast, Medical and Biological Centre, 97 Lisburn Road, Belfast, Northern Ireland, United Kingdom*
[3]*National Parks and Wildlife Service, 7 Ely Place, Dublin 2, Ireland*
[4]*Corresponding author: E-mail: emma.teeling@ucd.ie*

The recent identification of *Myotis brandtii* in Ireland raised the possibility that many roosts previously identified as *M. mystacinus* had the potential of being misidentified *M. brandtii*. Thus, the distribution and population estimates for *M. mystacinus* may have been over-estimated, while *M. brandtii* may have been under-estimated. Results from an all Ireland genetic survey of known *M. mystacinus* maternity roosts confirm that no long term misidentification has taken place. All specimens caught and sampled were *M. mystacinus*. Additonally, no further records of *M. brandtii* were found during six nights of woodland trapping using the acoustic lure. While the status of *M. mystacinus* in Ireland is now listed as 'least concern' in the Irish Red List, *M. brandtii* is listed as 'data deficient' and cannot currently be considered a resident species

Key words: Vespertilionidae, *Myotis*, cryptic species, Irish mammals

INTRODUCTION

Compared to continental Europe and Britain, Ireland has a relatively impoverished fauna, most likely resulting from its recent glacial history, small land area, island status and position at the north western edge of the European land mass. Currently, there are 34 terrestrial species of mammals in Ireland of which 10 are bats (Marnell *et al*., 2009). Over the last 12 years Ireland's species list has seen the addition of three new bat species. In 1997, two phonic types of *Pipistrellus pipistrellus*, were reclassified to *Pipistrellus pipistrellus* (common pipistrelle) and *P. pygmaeus* (soprano pipistrelle) (Barratt *et al*., 1997). *Pipistrellus nathusii* (Nathusius's pipistrelle) was confirmed to be breeding in Northern Ireland in 1998 (Russ *et al*., 1998) and most recently a few suspected *Myotis brandtii* (Brandt's bat) have been found (Kelleher, 2005; Harris 2006; Mullen, 2006).

Myotis brandtii is distributed throughout central and northern Europe, across Russia to the Urals, being sparsely distributed in Western Europe, the Mediterranean and the Balkans (Dietz *et al*., 2009). In Britain and mainland Europe, *M. brandtii* are associated with woodland and habitats near water (Harris and Yalden, 2008; Dietz *et al*., 2009), and roost in crevices in buildings or trees (Dietz *et al*., 2009). *Myotis brandtii* is a cryptic species, difficult to separate morphologically from *Myotis mystacinus* (whiskered bats), which are found across Europe including Ireland; *Myotis alcathoe* (Alcathoe's bat) and *Myotis aurascens* (steppe whiskered bat) which are found in continental Europe (Dietz *et al*., 2009). Separating *M. mystacinus* (whiskered bat) and *M. brandtii* based on morphology alone requires the use of unreliable characters such as the premolars (Berge, 2007) and penis shape (Harris and Yalden, 2008; Dietz *et al*., 2009). Genetically, these two species are quite distinct, with *M. brandtii* grouping with the New World *Myotis*, rather than the Old World *Myotis* which is typical of all other European myotid species (Ruedi and Mayer, 2001).

Myotis mystacinus is distributed throughout Europe and has a more southerly distribution than *M. brandtii*. They are associated with woodland, riparian and agricultural landscapes in Europe, and their nursery roosts are most frequently found buildings (Dietz *et al*., 2009). *Myotis mystacinus* has been known in Ireland since 1852 (Moffat, 1932). They are distributed across the island, yet prior to this study their conservation status was listed as indeterminate (Whilde, 1993). With only a few sightings and limited genetic identification, little is known about *M. brandtii* in Ireland, and therefore their status was unknown (Kelleher, 2005; Harris 2006; Mullen, 2006).

All of Ireland's bat species are protected under the Irish Wildlife Acts [1976 and 2000] and the Wildlife (Northern Ireland) Order 1985, and are listed in Annex IV of the European Union (E.U.) Habitats Directive [92/43/EEC]. The United Kingdom (UK) and Ireland are obligated under the E.U. Habitats Directive [92/43/EEC] and European Bat Agreement (Eurobats) to monitor and ensure favourable population status of all bat species. This requires documenting current population status and distribution of Irish bat species. Given the morphological similarity of *M. brandtii* and *M. mystacinus*, many roosts and records previously identified in Ireland as *M. mystacinus* have the potential of being mis- identified *M. brandtii* species. Thus, the distribution and population estimates for *M. mystacinus* may have been over-estimated while *M. brandtii* may have been under-estimated. We present results of an all Ireland survey to genetically confirm the presence of *M. mystacinus* and *M. brandtii* in an attempt to determine the status and distribution of these species in Ireland.

Materials and Methods

All 22 known maternity colonies of *M. mystacinus* (records from National Parks and Wildlife Service [NPWS] and Bat Conservation Ireland) were investigated (Fig. 1). Bats were caught using hand nets and harp traps during 2008 and 2009. Morphological identification of the bats was carried out using diagnostic features following Dietz and von Helversen (2004) and a tissue biopsy was taken from each wing. At one roost it was not possible to capture the bats however 10 faecal samples were taken from this site. Capture, biopsy and release of bats were performed under licenses issued by the NPWS and Northern Ireland Environment Agency. In 2009, surveys of woodlands using an acoustic lure (Hill and Greenaway, 2005) were carried out in two woodlands where suspected *M. brandtii* had been previously caught, Glendalough, County Wicklow, and Killarney National Park, County Kerry, one the largest areas of native woodland in Ireland Cabot (1999) (see Fig. 1).

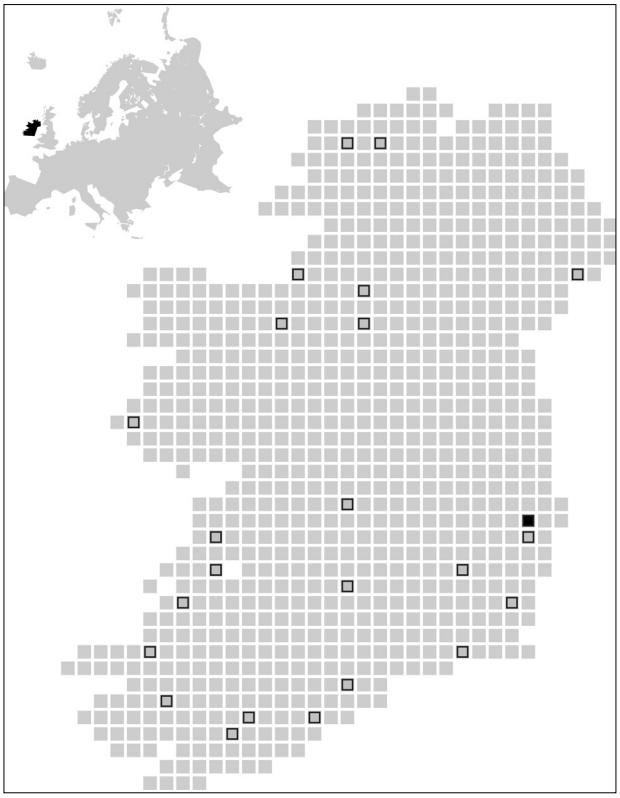

Fig. 1. Gridmap of Ireland (10 × 10 km squares) plotted by IrishGrid v0.4 (Bekaert, 2009). Locations of confirmed *M. brandtii* records (■, Glendalough, County Wicklow) and known maternity roosts of *M. mystacinus* sampled (□)

A 'DNA barcoding' technique was used for species confirmation. We used the 5' end of the Cytochrome C oxidase Subunit 1 mitochondrial region (COI — Ratnasingham and Hebert, 2007). DNA extractions from tissue and faecal samples were carried out using DNeasy and QIAamp DNA Stool Mini kits (Qiagen) respectively. PCR amplifications of the COI region were performed with 2 µmol/l of each primer (forward, ATACTTCGGGGTGGCCGAAGAATCA; reverse, TYTCAAC CAAYCACAAAGATATYGG) labelled with an M13 tail, 1.5 mmol/l $MgCl_2$, 1 U of Platinum Taq DNA polymerase (Invitrogen), and 10 ng of DNA. The touchdown PCR reaction consisted of: denaturation at 95°C for 3 min, 10 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s minus 1°C per cycle, extension at 72°C for 60 s; followed by 35 cycles with 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 60 s. The PCR products were separated and visualised in a 1% agarose gel. All PCR products were sequenced in both directions with M13 primers, assembled on Sequencher v4.9 (Gene Codes Corporation).

Samples of known *M. mystacinus* (20 Germany four France) and *M. brandtii* (two German) were included, along with 11 individuals from four other representative European *Myotis* spp. (one *M. emarginatus*, Italy; two *M. daubentonii*, France; two *M. nattereri*, UK; two *M. bechsteinii*, France; four *M. alcathoe*, France) to demonstrate the phylogenetic relationships and establish the genetic identification of the sampled bats. One, *Nyctalus leisleri* was included in the analysis as an outgroup for phylogenetic reconstructions. The sequences were aligned using ClustalW v2.0.12 (Larkin *et al*., 2007) and then

TABLE 1. COI species haplotypes, GenBank accession numbers, county of origin with number of samples sequenced in parentheses and sample collector

Haplotype	Accession No.	Origin	Collector
M. emarginatus	GU270553	IT (1)	J. Altringham and C. Jan
M. daubentonii	GU270554	FR (2)	S. Puechmaille
M. mystacinus H1	GU270555	IE (107), FR (4), DE (19)	Current study; S. Puechmaille; C. Dietz
M. mystacinus H2	GU270556	IE (38)	Current study
M. mystacinus H3	GU270557	IE (1)	Current study
M. mystacinus H4	GU270558	DE (1)	C. Dietz
M. mystacinus H5	GU270559	FR (1)	S. Puechmaille
M. alcathoe	GU270560	FR (1)	S. Puechmaille
M. nattereri	GU270561	UK (2)	J. Altringham and C. Jan
M. bechsteinii H1	GU270562	FR (1)	S. Puechmaille
M. bechsteinii H2	GU270563	FR (1)	S. Puechmaille
M. brandtii H1	GU270564	DE (1)	J. Altringham and C. Jan
M. brandtii H2	GU270565	DE (1)	J. Altringham and C. Jan
N. leisleri	GU270566	FR (1)	S. Puechmaille

collapsed into haplotypes (Table 1) using FaBox v1.35 (Villesen, 2007). The generated sequences were deposited in GenBank (Table 1).

Maximum likelihood (ML), minimum evolution (ME) and parsimony were used to reconstruct phylogenetic relationships and confirm species identity using PAUP v4.0b10 (Swofford, 1991). Modeltest v3.7 (Posada and Crandall, 1998) was used to estimate the most suitable model of sequence evolution [GTR + Γ; Base pair frequencies = (0.3409, 0.1243, 0.2388); *R*-matrix = (1.0000, 24.3779, 1.0000, 1.0000, 18.7990); Shape parameter of gamma distribution = 0.1351)]. All searches were performed using heuristic searches with tree-bisection and recombination branch swapping. Starting trees were obtained via Neighbor-joining in ML and ME analyses. One hundred bootstrap replicates were performed for ML and 1000 for ME and parsimony (Fig. 2).

RESULTS

One hundred and forty-five bats from 22 suspected *M. mystacinus* maternity roosts across Ireland were sampled (Fig. 1). Over six night's woodland sampling using the acoustic lure, 10 bats were caught, representing six species, including *Plecotus aurtius*, *Nyctalus leisleri*, *P. pygmaeus*, *M. daubentonii*, *M. nattereri*, and single *M. mystacinus*/*brandtii* in Glendalough, County Wicklow.

All individuals were morphologically identified as *M. mystacinus* according to dentition and penis shape. A 556 bp section of the COI gene was examined. There were 76 parsimony informative sites between *M. mystacinus* and *M. brandtii* haplotypes. All phylogenetic analyses placed the 146 *M. mystacinus*/*brandtii* sequences into a well supported (100% bootstrap support all analyses) monophyletic clade of *M. mystacinus*, with no *M. brandtii*, confirming that the sampled bats were all *M. mystacinus* (Fig. 2). In total, five COI haplotypes of *M. mystacinus* were identified (Table 1). The most common *M. mystacinus* haplotype occurred in samples from

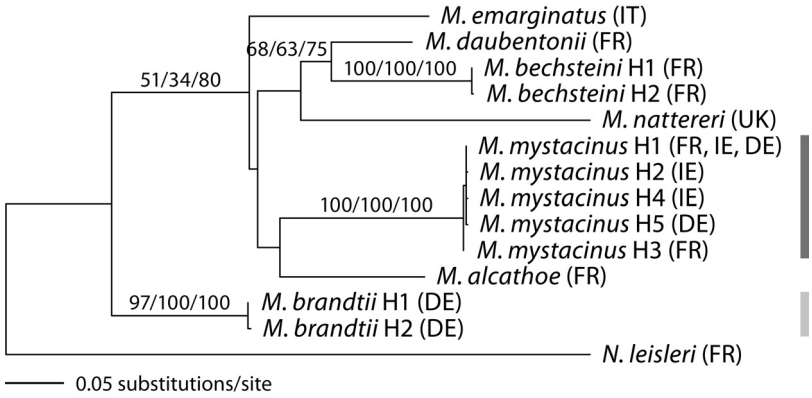

FIG. 2. Maximum likelihood tree (lnL = -1753.7) of 556 bp fragment of COI. Bootstrap values from maximum likelihood, distance and parsimony are shown in this order above branches

Ireland, Germany and France. While two haplotypes were found only in Ireland samples, one of which was unique to a single sample. One was unique to a German sample and another to a French sample (Table 1). All known *M. brandtii* sequences formed a well supported monophyletic clade (Fig. 2).

Discussion

The island-wide genetic survey of all known *M. mystacinus* roosts, confirmed that there has not been a long-term misidentification of *M. brandtii* as *M. mystacinus* in Ireland. This study showed that *M. mystacinus* is widespread throughout Ireland, and as a result has been listed as 'least concern' in the Irish Red List (Marnell *et al*., 2009). Yet, further research is needed to elucidate their ecology in Ireland and the distribution of genetic diversity to fully confirm this.

The woodland survey in Killarney National Park and Glendalough, provided no further *M. brandtii* records. Thus the Irish Red List (Marnell *et al*., 2009) status for *M. brandtii* has been recorded as 'data deficient'. These results suggest that *M. brandtii* are rare and possibly endangered in Ireland. Little is known about their ecology, but they are generally thought to be associated with broadleaf woodland (Taake, 1984). Approximately 1% of Ireland is covered in native deciduous woodland due to historical deforestation (Perrin *et al.*, 2008). If *M. brandtii* is reliant on this habitat then this could limit its distribution in Ireland, analogous to the situation with *M. bechsteinii* in Britain (Harris and Yalden, 2008). It is possible that *M. brandtii* has been resident in Ireland since the early Holocene and the destruction of Irish forests may have caused a major decrease in its distribution and abundance. Alternatively, the recent confirmed records of *M. brandtii* in Ireland may have been vagrants. Since no breeding colony has been found we cannot confirm this species is a resident breeder. However, *M. brandtii* is not thought to be a long distance migrant in Europe (Dietz *et al*., 2009) and therefore suggests that this hypothesis is untenable.

The present results may also reflect our survey approach. We targeted known *M. mystacinus* roosts, which are biased towards the larger maternity roosts in dwellings. These sites may not reflect the full range of roost types available. *Myotis brandtii* has been found to roost in trees, bat boxes and buildings (Sachanowicz and Ruczyński, 2001; Dietz *et al*., 2010). Yet, differences in building architecture across Europe may produce intraspecific variation in roost usage across a species range (Marnell and Presetnik, 2010). If *M. brandtii* utilise roost sites differently than *M. mystacinus* in Ireland, for example, primarily selecting tree roosts, they could have been missed in the present survey. This would also explain the conspicuous absence of *M. brandtii* roost records to date. Currently there has been no comprehensive study of the usage of tree roosts by Irish bats (McAney, 2006). Additionally, our limited woodland surveys using an acoustic lure and mist nets proved unsuccessful in catching *M. brandtii*, however, catch rates were low for all species. This study, confirmed the distribution of *M. mystacinus* in Ireland, however, more research needs to be done to investigate the occurrence *M. brandtii*, whether this be through more intensive sampling or through new survey techniques.

Acknowledgements

We thank Sébastien Puechmaille, John Altringham, Camille Jan and Christian Dietz for tissue samples, National Parks and Wildlife Service regional staff and roost owners for help and co-operation. This project was funded by the NPWS, the Heritage Council and the Irish Research Council for Science Engineering and Technology.

Literature Cited

Barratt, E. M., R. Deaville, T. M. Burland, M. W. Bruford, G. Jones, P. A. Racey, and R. K. Wayne. 1997. DNA answers the call of pipistrelle bat species. Nature, 387: 138–139.

Bekaert, M. 2009. IrishGrid v0.4. http://irishgrid.googlecode.com. Accessed 10 December 2009.

Berge, L. 2007. The effects of phylogenetic differences on resource partitioning between the cryptic species whiskered bat (*Myotis mystacinus*) and Brandt's bat (*M. brandtii*). Unpublished Thesis, University of Bristol, School of Biological Sciences, Bristol, UK, 162 pp.

Dietz, C., and O. von Helversen. 2004. Illustrated identification key to the bats of Europe. Version 1.0. http://www.mammalwatching.com/Palearctic/Otherreports/batkey.pdf, accessed 9th December 2010.

Dietz, C., O. von Helversen, and D. Nill. 2009. Bats of Britain, Europe and northwest Africa. A and C Black, London, UK, 400 pp.

Harris, S. 2006. Generation of a species-specific DNA sequence library of UK British bats. Iternational report. The Joint Nature Conservation Committee and Veterinary Laboratories Agency, 20 pp.

Harris, S., and D. W. Yalden. 2008. Mammals of the British Isles: handbook, 4th edition. Mammal Society, Southampton, UK, 800 pp.

Hill, D. A., and F. Greenaway. 2005. Effectiveness of an acoustic lure for surveying bats in British woodlands. Mammal Review, 35: 116–122.

Kelleher, C. 2005. Bat fieldcraft workshop. A report to the National Parks and Wildlife Service. Killarney National Park, Killarney, County Kerry, 23 pp.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23: 2947–2948.

Marnell, F., and P. Presetnik. 2010. Protection of overground roosts for bats (particularly roosts in buildings of cultural heritage importance). EUROBATS Publications Series No. 4 (English version), UNEP/EUROBATS Secretariat, Bonn, 57 pp.

Marnell, F., N. Kingston, and D. Looney. 2009. Terrestrial mammals. Ireland Red List, 3: 1–50.

McAney, K. 2006. A conservation plan for Irish vesper bats. Irish Wildlife Manual, 20: 1–57.

Moffat, C. B. 1932. The whiskered bat. The Irish Naturalist's Journal, 4(6): 106–109.

Mullen, E. 2006. Brandt's bat *Myotis brandtii* in Co. Wicklow. Irish Naturalist Journal, 28: 343.

Perrin, P., J. Martin, S. Barron, F. O'Neill, K. McNutt, and A. Delaney. 2008. National survey of native woodlands. Unpublished Report to the National Parks and Wildlife Service. National Parks and Wildlife Service, Dublin, 187 pp.

Posada, D., and K. A. Crandall. 1998. ModelTest: testing the model of DNA substitution. Bioinformatics, 14: 817–818.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: the barcode of life data system. Molecular Ecology Notes, 7: 355–364.

Ruedi, M., and F. Mayer. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Molecular Phylogenetics and Evolution, 21: 436–448.

Russ, J. M., J. K. O'Neill, and W. I. Montgomery. 1998. Nathusius' pipistrelle bats (*Pipistrellus nathusii*, Keyserling and Blasius 1839) breeding in Ireland. Journal of Zoology (London), 245: 345–349.

Sachanowicz, K., and I. Ruczyński. 2001. Summer roosts sites of *Myotis brandtii* (Eversmann, 1845) (Chiroptera, Vespertilionidae) in eastern Poland. Mammalia, 65: 531–535.

Swofford, D. L. 1991. PAUP: phylogenetic analysis using parsimony. Illinois Natural History Survey, Champaign, IL.

Taake, K. H. 1984. Strukturelle unterschiede zwischen den sommerhabitaten von kleiner und grosser bartfledermaus *Myotis mystacinus* und *Myotis brandti* in Westfalen. Nyctalus, 2: 16–32.

Villesen, P. 2007. FaBox: an online toolbox for fasta sequences. Molecular Ecology Notes, 7: 965–968.

Whilde, A. 1993. Threatened mammals, birds, amphibians and fish in Ireland: Irish Red Data Book 2: Vertebrates. HMSO, Belfast.

Received 15 December 2009, accepted 02 August 2010