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Barcoding reveals the first record of *Lamyctes africanus* (Porath, 1871) in Germany (Chilopoda: Lithobiomorpha)

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Abstract. The henicopid centipede *Lamyctes africanus* (Porath, 1871) is recorded for Germany for the first time. The species was first discovered by barcoding methods and subsequently confirmed by morphological characters, and specimens were also collected from additional locations. Information about the German sites where *L. africanus* was found is given and its mode of dispersal is discussed.

Keywords. Barcode, COI, Arthropoda, alien species, dispersal

INTRODUCTION

The German Barcode of Life project (GBOL) aims to build up a DNA library of organisms occurring in Germany with a corresponding voucher and DNA collection. Further goals are to develop DNA barcode applications with benefits for e. g. species inventory or monitoring purposes. The GBOL project Myriapoda barcoded nearly all ca. 200 indigenous Diplopoda and Chilopoda species of Germany (Voigtländer et al. 2011) as well as specimens and species from other European countries. While in most cases the molecular phylogeny of the present GBOL data agrees with the current view of taxonomy, in some groups, especially in Chilopoda, it points out taxonomic problems and confusion (Wesener et al. 2015, 2016).

Some centipedes are not indigenous to the fauna of Germany, but could become established as alien species and disperse naturally or by human influence to synanthropic or natural habitats; additional species only occur in greenhouses (Decker et al. 2014). While the centipede family Lithobiidae is the dominant family of centipedes of the order Lithobiomorpha in Europe, the family Henicopidae is a typical southern hemisphere group, represented in Europe mainly by a few species of the genus *Lamyctes* Meinert, 1868, apart from two species of other genera (Christian & Szeptycki 2004; Kurochkina 2007). Currently, five species of *Lamyctes* are known for Europe. *Lamyctes emarginatus* (Newport, 1844) is a subcosmopolitan, frequently recorded species from many European countries (Enghoff et al. 2013; Zapparoli 2010), which is found in anthropogenic as well as natural habitats. It is a typical pioneer species, combining a fast life cycle, reaching maturity 6 to 12 weeks after hatching, with parthenogenetic reproduction in Europe (Zerm 1997). In Central Europe this species is mostly found in floodplains, disturbed habitats, urban areas and in agricultural land, with a preference for wet habitats with low vegetation cover (Voigtländer 2005). The taxonomic status of *L. emarginatus* and its synonymy with *L. fulvicornis* Meinert, 1868 remains doubtful (Enghoff et al. 2013). The subcosmopolitan *Lamyctes coeculus* (Brölemann, 1889) seems to be restricted to greenhouses in Europe and is presently known from Denmark, Finland, France, Germany, Great Britain, Italy and Sweden (Barber 2009; Decker et al. 2014; Edgecombe & Giribet 2003a; Enghoff 1975). Only a few records exist for *Lamyctes albipes* (Pocock, 1894) from the Canary Islands (as *L. mauriesi* in Eason & Enghoff 1992), and *L. inexpectatus* Kurochkina, 2007 from the European part of Russia (Kurochkina 2007). The latest addition to the European alien species fauna is *Lamyctes africanus* (Porath, 1871), found in outdoor habitats as a pioneer species in Denmark (Enghoff et al. 2013), and recently found in greenhouses in Edinburgh, Great Britain (Barber, 1992) and Olomouc, Czech Republic (Dányi & Tuf 2016) as well as in flowerpots from a garden in Arles, France (Iorio 2016). Enghoff et al. (2013) present a detailed review of *L. africanus* with a full redescription and comparison with the similar *L. emarginatus*, as well as a key to all the *Lamyctes* species in Europe other than *L. inexpectatus*.

There have been few barcoding and molecular phylogenetic studies on Henicopidae (Edgecombe et al. 2002;

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Edgecombe & Giribet 2003a, b), and Enghoff et al. (2013) have found that the only *L. africanus* specimen sequence deposited on Genbank in fact represents an undetermined species. One of the Genbank sequences from the same original study (Edgecombe et al. 2002; Edgecombe & Giribet 2003a, b) available for *L. emarginatus* was genetically almost identical to the Danish *L. africanus*, distinct from other *L. emarginatus*, and impossible to verify because the voucher specimen from Australia was damaged.

In Germany, *L. emarginatus* occurs frequently (Voigtländer 2005) and *L. coeculus* has been found in the greenhouses of the Leipzig Botanical Garden and the Berlin-Dahlem Botanical Garden (Decker et al. 2014).

The origin and native distribution area of all introduced *Lamyctes* species remain doubtful due to the subcosmopolitan dispersal, the mostly synanthropic records and the lack of data of natural habitats from likely areas of origin such as, for example, South Africa and Australia.

Here, we present the first record of *L. africanus* from Germany, as well as a discussion of how the data on German and Austrian *Lamyctes* fits into the worldwide barcoding data available for the genus.

MATERIAL AND METHODS

Specimen collection and preparation

Our sample included nine specimens of *Lamyctes* from three localities in Germany (see Table 1). This comprises the two species (*L. emarginatus*, *L. coeculus*) previously known and the first record of *L. africanus* in Germany.
All specimens are stored as vouchers in 95% undenatured ethanol, either at the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK) or the Zoologische Staatssammlung München, Germany (ZSM) (see Table 1).

Specimens used in the study were collected by hand and transferred to vials containing 95% undenatured ethanol within days of collection. The vials contain an individual GBOL number with which the specimens can be connected to the accompanying data. After conservation the specimens were sent to the GBOL facility at the ZFMK. Upon arrival, all specimens were photographed (images are or will be uploaded to BOLD, http://www.boldsystems.org/), and a tissue sample was removed for DNA extraction. For this specific GBOL subproject, DNA extraction was attempted for nine specimens of *Lamyctes* (see Table 1). DNA extraction and sequencing protocols follow those of earlier studies (Wesener et al. 2015, 2016).

After the first discovery of *L. africanus* in our GBOL-Myriapoda project, more material of *L. emarginatus* from more than 75 localities in Germany (mostly North Rhine-Westphalia, Saxony, Saxony-Anhalt and some other federal states) with >350 specimens, all deposited in the collection of Senckenberg Museum of Natural History Görlitz (SMNG) and the private collection of N. Lindner (coll. Lindner), were checked by P. Decker, K. Voigtländer and N. Lindner for potential confusion with *L. africanus* using the redescription and key provided in Enghoff et al. (2013).

Illustrations

The final phylogenetic tree was edited using Adobe Illustrator CS4. The map was created with ArcGIS 10.

Phylogenetic analysis

Sequences were obtained for nine *Lamyctes* specimens. The available sequences of ten further *Lamyctes* as well as *Henicops maculatus* Newport, 1845, *H. brevilabiatus* (Ribaut, 1923) and *Lithobius forficatus* (Linnaeus, 1758) as outgroups were downloaded from GenBank and added from previously published datasets (Edgecombe et al. 2002; Edgecombe & Giribet 2003a, b; Enghoff et al. 2013). Because of the uncertain identity of one of the *L. africanus* and *L. emarginatus* sequences downloaded from Genbank, as noted by other authors (Enghoff et al. 2013), the specimen names were put in quotation marks.

Sequences were aligned by hand in Bioedit (Hall 1999). The final dataset included 22 nucleotide sequences with 657 positions. Phylogenetic analyses were conducted in MEGA6 (Tamura et al. 2013). Modeltest, as implemented in MEGA6 (Tamura et al. 2013), was performed to find the best fitting maximum likelihood substitution model. Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the best substitution pattern. Included codon positions were 1st+2nd+3rd+Noncoding. Modeltest selected the General Time Reversible model (Nei & Kumar 2000) as best fitting model. The tree with the highest log likelihood (-4725.2866) is used here to infer the genetic distances and evolutionary history of the analyzed specimens. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7010)). The rate variation model allowed for some sites to be evolutionarily invariable ((+I), 45.2% sites). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the analyzed taxa. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Table 1. GBOL numbers, GenBank codes and locality data. GBOL number refers to DNA extraction and BOLD registration. ZFMK = Zoological Research Museum A. Koenig, Bonn, Germany; ZSM = Zoologische Staatssammlung München, Germany.

Species	GBOL	GenBank	Voucher	Locality
Lamyctes coeculus	ZFMK-TIS-1420	KM491571	ZFMK MYR3656	Germany, Saxony, Leipzig Botanical Garden, greenhouse
Lamyctes coeculus	ZFMK-TIS-2519811	KM491619	ZFMK MYR3807	Germany, Saxony, Leipzig Botanical Garden, greenhouse
Lamyctes coeculus	ZFMK-TIS-2538271	KX442653	ZFMK MYR5136	Germany, Mecklenburg-Western Pomerania, Bansin, greenhouse
Lamyctes africanus	ZFMK-TIS-2538242	KX442652	ZFMK MYR5137	Germany, Saxony-Anhalt, Gerwisch
Lamyctes emarginatus	ZFMK-DNA-100410134	KX442654	ZSM-JSP120527-016	Germany, Bavaria, Freising
Lamyctes emarginatus	ZFMK-DNA-100410146	KX442655	ZSM-JSP120527-014	Germany, Bavaria, Freising
Lamyctes emarginatus	ZFMK-DNA-112780067	KX442656	ZSM-JSP120530-007	Germany, Bavaria, Freising
Lamyctes emarginatus	ZFMK-DNA-112780068	KX442657	ZSM-JSP120527-008	Germany, Bavaria, Freising
Lamyctes emarginatus	ZFMK-DNA-112780091	KX442658	ZSM-JSP120527-008	Germany, Bavaria, Freising

Distance analysis

The number of base differences per site between sequences is shown. The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 657 positions in the final dataset. Evolutionary distance analyses were conducted in MEGA6 (Tamura et al. 2013).

RESULTS

Molecular analysis

Monophyly of *Lamyctes* is not supported here by the molecular data (Fig. 1), as *Henicops maculatus* branches within *Lamyctes* and forms a sister clade with *L. inermipes* (Silvestri, 1897) with low bootstrap support (43%).

H. brevilabiatus, *H. maculatus*, *L. hellyeri* Edgecombe & Giribet, 2003, *L. inermipes* and the *Lamyctes* "*africanus*" specimen from Cape Town, South Africa, form distinct lines.

The *L. africanus* clade is well supported (100%) and the sequence of the German specimen from Gerwisch is identical to the *Lamyctes* "*emarginatus*" from a garden in Sydney, Australia, as well as differing only in one base pair from the Danish *L. africanus* specimen (Fig. 1).

The monophyly of *L. coeculus* is well supported (100%). The German specimens from the Leipzig Botanical Garden have identical sequences with the specimen from Argentina and a forest northwest of Sydney, while the specimen from a small greenhouse in Bansin, Germany, differs in a single base pair from the other specimens.

L. emarginatus forms a well supported branch (100%) with two lineages, grouping as a German-Canadian clade in opposition to a purely Canadian clade, both well supported (100%).

Intraspecific distances vary within *L. coeculus* (0–0.3%), *L. emarginatus* (0–9.6%) and *L. africanus* (0–0.8%). Distances between species ranges from 14.5% (*L.* "*africanus*" to *L. hellyeri*) to 19.3% (*L. emarginatus* to *L. coeculus*), while distances to the outgroup species *H. brevilabiatus*, *H. maculatus* and *L. forficatus* ranges from 15.6% to 24.4%.

Records of *L. africanus* in Germany

In Germany, *L. africanus* was found at seven localities (Fig. 2), often sympatrically with *L. emarginatus*. No males were found. All specimens fit the descriptions by Porath (1893, 1894) and the redescriptions by Enghoff et al. (2013) and Iorio (2016).

Material examined. North Rhine-Westphalia: Truppenübungsplatz Haltern-Borkenberge, open sandy dry meadow with *Filago minima*, 51.767°N 7.299°E, 17.XII.2006, 06.V.2007, pitfall trap, leg. K. Hannig (SMNG VNR017096, 017097), 17 females; Ascheberg (West-

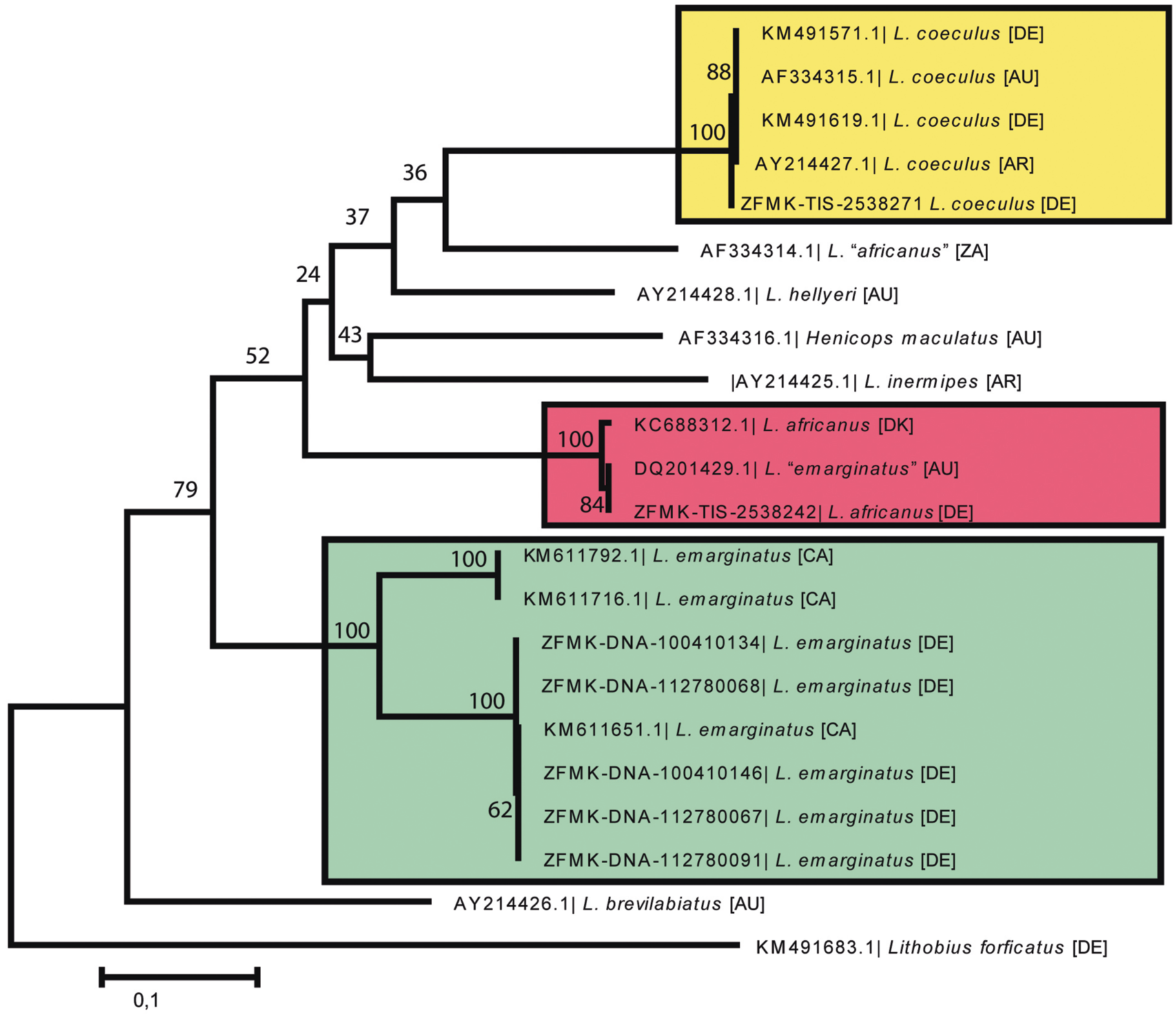

Fig. 1. Maximum likelihood tree under the GTR model, 1000 bootstrap replicates, for *Lamyctes*. Country of origin given in square brackets: AR = Argentinia; AU = Australia; CA = Canada; DE = Germany; DK = Denmark; ZA = South Africa. For full data on all specimens, see Table 1.

falen), railway station, fallow land, 51.783°N 7.601°E, 20.IX.2015, hand collecting, leg. K. Hannig (SMNG VNR017286), 2 females; Arnsberg-Bachum, bank of Ruhr River, gravel, 51.469°N 7.932°E, 15.VIII.2008, hand collecting, leg. K. Hannig (SMNG VNR017098), 3 females; Saxony: Leipzig, fallow land beside an abandoned railway area; 51.342°N 12.388°E; 24.IV.2008, leg. N. Lindner (coll. Lindner 2009-CL-3603), 1 female; Saxony-Anhalt: Gerwisch (near Magdeburg), inland dune, 52.187°N 11.723°E, 28.VIII.2014, 28.IX.2014, 01.X.2014, hand collecting (1x), leg. N. Lindner & pitfall trap (2x), leg. Förder- und Landschaftspflegeverein Biosphärenreservat "Mittelelbe" (FÖLV) (coll. Lindner 2014-CL-8325, 2014-CL-8682, 2014-CL-8683), 4 females; Wartenburg (near Lutherstadt Wittenberg), orchard meadow within the open floodplain of the Elbe river, 51.809°N 12.757°E, 30.X.2012, 18.X.2012, 4.XII.2012, 13.IX.2013, 26.IX.2013, hand collection (1x), leg. N. Lindner & pitfall trap, leg. FÖLV (coll. Lindner 2012-CL-6086, 2016-CL-6299, 2013-CL-6695, 2014-CL-7311, 2014-CL-7312), >10 females; Heudeber (near Wernigerode), orchard meadow, 51.908°N 10.854°E, 14.VIII.2013, pitfall trap, leg. FÖLV (coll. Lindner 2014-CL-7279), 1 female.

DISCUSSION

Molecular analysis

The *L.* "*emarginatus*" specimen from a garden in Sydney, Australia, branching within *L. africanus*, is here confirmed again genetically as *L. africanus*, showing a 100% identity with our German *L. africanus*. Enghoff et al. (2013) examined the fragment of a specimen (AMS KS5760) by

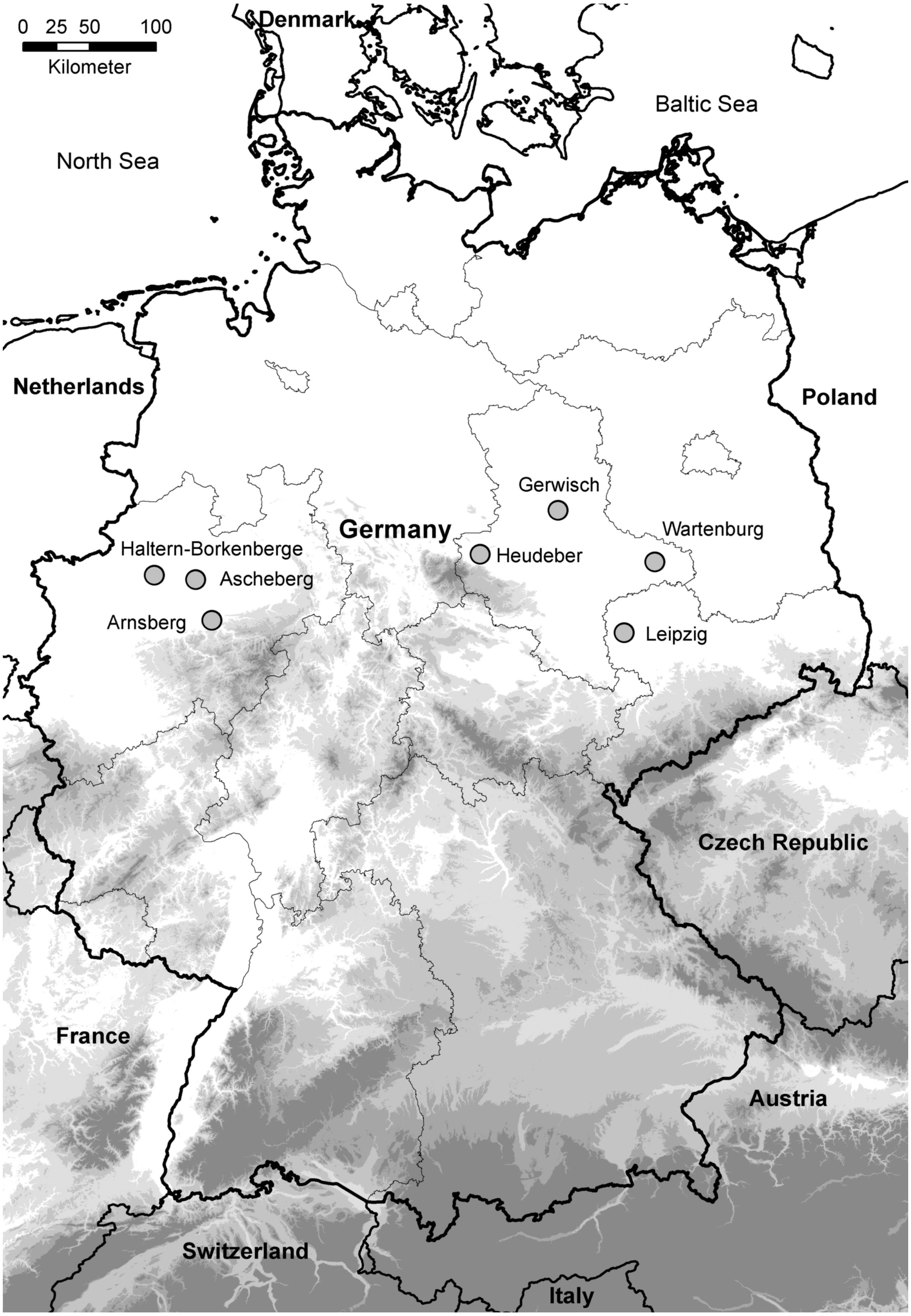

Fig. 2. Records of *Lamyctes africanus* (grey circles) in Germany.

mistake as the voucher of DQ201429.1. However, the original voucher collected by G. Edgecombe at the same locality in the Australian Museum Garden on the 3rd November 2004 (MCZ 131488, DNA103924) was also examined, but as it was missing legpairs 10 to 15, it could also not be identified as either *L. emarginatus* or *L. africanus* (G. Edgecombe, pers. comm.). The *L.* "*africanus*" from South Africa also forms a distinct branch in this study, suggesting a different species. It also differs from *L. emarginatus* and *L. africanus* in morphology (Enghoff et al. 2013).

Mysterious origin of Central European Lamyctes

The German *L. africanus*, because of its identical COI sequence, could have been introduced from the same source population as the specimen from a garden in Sydney. This species was also recorded from some localities in southwest Australia (Attems 1911). As the European outdoor records are scattered along the plain of the northern half of Germany and equally scattered in Denmark (Enghoff et al. 2013: Fig. 4) it can be assumed, that the species had been introduced a much longer time ago and might be widely distributed now, but until recently has been always confused with *L. emarginatus*. This has already been pointed out by Enghoff et al. (2013).

It is not clear if the Danish specimens of *L. africanus* might originate from a different source or if the slight differences in the sequences are caused by errors during sequencing. There is still no evidence that this species is indigenous to Australia.

The *L. coeculus* from the hothouse in Leipzig are probably also from the same original source as the ones from Australia and Argentina. Whether the Australian population, collected in a *Eucalyptus*-*Acacia* forest in New South Wales, represents a natural population or one originating from elsewhere is not clear.

While the two clustering specimens of *L. emarginatus* from Canada are from one original source, the German and another *L. emarginatus* from Canada probably originate from the same haplotype. All German samples have been collected from a single river bank in Freising, Bavaria (Isar Valley), thus representing a single population. They need to be checked with more, geographically remote samples to see if *L. emarginatus* shows a wider genetic variability or represents a single haplotype within Germany.

More sampling in natural habitats in Australia could clarify whether genetic diversity is higher in this area and whether males occur there, which could provide evidence that both species are native to Australia. For *L. emarginatus*, specimens from the type localities in Jutland and on islands in Denmark (*L. fulvicornis*) and New Zealand (*L. emarginatus*) could reveal the origin and status of this subcosmopolitan species.

Dispersal not only by train?

In Denmark *L. africanus* was always recorded at abandoned railway areas, implying a dispersal by train. In Germany there have been, up to now, two records from a similar habitat – specimens were found in Leipzig at a site with sparse vegetation near a railway area and in fallow land at the railway station in Ascheberg.

The record from Gerwisch is from the edge of a dune near the River Elbe; an additional record is from an orchard meadow in Wartenburg, where the River Elster flows into the Elbe. Both sites have been flooded regularly in the past, for example, during the last large-scale flooding in 2013. There were no specimens of *L. africanus* found in other material from numerous other eastern German floodplains investigated, where *L. emarginatus* has been recorded, on the River Elbe, River Mulde, River Zwickau Mulde, River Freiberger Mulde, River Havel and River Lusatian Neisse. Possibly *L. africanus* has become only recently established at the site. In addition, *L. africanus* was also found in river gravel on the River Ruhr in western Germany. Hence, dispersal through water and floating material could be a possible source and way for dispersal of this species. Also a similar submersion resistance of eggs in *L. africanus* to that recorded in *L. emarginatus* (Zerm 1997; Zulka 1992) may be assumed.

Another record of *L. africanus* is from a sparsely vegetated dry meadow in a military training ground. Originally all specimens were identified in error as *L. emarginatus* (Decker et al. 2009), but both species co-occur at this site. The Haltern-Borkenberge Training Area has been used as a (military) training area and shooting range since 1873 and has been under the command of the British Army from 1945 to May 2015, but was also used by the German, Belgian, and Dutch Armies. The open sandy site with pioneer vegetation, where *L. africanus* was recorded, resulted from tracked and wheeled vehicles, mowing, annual vegetation burning and explosive devices (see also Olthoff et al. 2009; Zimmermann & Feuring 2009). This training area is not accessible to the public and the most likely source of introduction of *L. africanus* could be military vehicles.

The record from Heudeber was also from an orchard meadow, as in Wartenburg, but the site had not been flooded by a river in the past.

L. africanus has not, as yet, been found in very anthropogenic influenced urban habitats such as gardens, parks, reclaimed mine sites or colliery spoil heaps in Germany. However, this species prefers open sandy to gravel habitats with sparse vegetation.

Finally, there seem to be several likely ways of dispersal at least for *L. africanus*: by train or along railways, military vehicles, and along rivers through flooding. Enghoff et al (2013) also mentioned a specimen of *Lamyctes* in a flowerpot from Annemasse in France, which could resemble *L. africanus*. Recently, *L. africanus* was actually confirmed from a flowerpot in Arles, France (Iorio 2016).

We would predict that the number of records of this (overlooked) new alien *Lamyctes* species in Europe or elsewhere will increase rapidly in the future, as also because of its current progress of dispersal, especially if it is dispersed with flowering plants and along railways.

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