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# Barcoding pest species in a biodiversity hot-spot: the South African polyphagous broad-nosed weevils (Coleoptera, Curculionidae, Entiminae)

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## Abstract

Polyphagous broad nosed weevils (Curculionidae: Entiminae) constitute a large and taxonomically challenging subfamily that contains economically significant agricultural pests worldwide. South Africa is a hot-spot for biodiversity and several species of indigenous and endemic genera of Entiminae have shifted onto cultivated plants, with some being phytosanitary pests. The sporadic nature of many species, and the presence of pest complexes and cryptic species presents an identification challenge to non-specialists. Furthermore no comprehensive identification tools to identify immature stages that may be found in crops/soil exist. In this paper a curated barcoding database with 70 COI sequences from 41 species (39 Entiminae, 2 Cyclominae) is initiated, to assist with the complexity of identification of species in this group.

## Keywords

Entiminae, PBNW, agricultural pests, identification, COI barcode

## Introduction

Curculionidae are a hyperdiverse family of beetles (Coleoptera), containing approximately 17 subfamilies and more than 51 000 described species (Oberprieler et al. 2007, Bouchard et al. 2011, Leschen and Beutel 2014). Curculionidae are predominantly phytophagous, and as a family utilize every organ of practically every higher plant species. Within the Curculionidae, the Entiminae is the largest subfamily, containing approximately 53 tribes, 1370 genera and more than 12 000 described species (Oberprieler et al. 2007, Bouchard et al. 2011, Marvaldi et al. 2014). Entiminae are usually small to medium sized weevils (4-30 mm), with short, broad rostrums usually not more than twice as long as wide

(Marvaldi et al. 2014). The adults have deciduous mandibular cusps that are used to dig their way out of soil after pupation, and are then actively lost while feeding, leaving a distinctive scar (Thompson 1992, Marvaldi 1997, Marvaldi and Lanteri 2005). Eggs are deposited in plant material and debris, or soil, without rostrum participation (Howden 1995, Marvaldi 1997), and the apodous, C-shaped larvae feed externally on plant roots, eventually pupating in soil (Marvaldi 1997, Marvaldi et al. 2014). Most entimine weevil species have loose host plant associations, being oligo-or-polyphagous in larval and adult stage; several species constitute some of the most economically important agricultural pests worldwide (Oberprieler et al. 2007, Marvaldi et al. 2014). Larval feeding damages roots; while adults feeding damages leaves, shoots and fruits of their host plants, typically causing a notching pattern on leaves and shallow craters and/or scarring on fleshy tissue like fruits (Marvaldi et al. 2014, Prinsloo and Uys 2015).

South Africa has a rich native fauna of Entiminae, notably with considerable diversity in the tribes Oosomini, Embrithini and Tanyrhynchini (Alonzo-Zarazaga and Lyal 1999, Oberprieler 1995, Oberprieler 1988, Borovec and Oberprieler 2013). The Entiminae includes agricultural pest species that have been recorded on every group of cultivated plants in the country (Marshall 1920, Marshall 1927, Marshall 1939, Anneck and Moran 1982, Oberprieler 1988, Prinsloo and Uys 2015). Many of the species recorded seem to be sporadic pests; despite having been recorded in the previous century by Marshall they do not feature as regularly occurring major pests of that crop (Prinsloo and Uys 2015). Many native entimine pests (hereafter called 'polyphagous broad nosed weevils', PBNW) probably shifted opportunistically from native vegetation onto a cultivated host as agriculture encroached on natural ecosystems, a process that's been deduced from pest appearance *Eremnus cerealis* Marshall, 1921 (Entiminae:Tanyrhynchinae) and *Oosomus varius* Boheman, 1843 (Entiminae:Oosomini) on small grains and vines, and forestry pines (*Pinus radiata* D. Don) respectively in the South Western Cape of Southern Africa (Pettey 1922, Tribe 1991). Some species like the banded fruit weevil, *Phlyctinus callosus* Schoenherr, 1826 (Entiminae: Oosomini) are able to complete their life-cycle in cultivated habitats (orchards and vineyards in South Africa); with larvae and adults feeding on crop and weeds and presenting a problem in same area every growing season (Barnes 1987, Fisher and Learmonth 2003, Pryke and Samways 2007). In some cultivated habitats, like orchards and vineyards in the Western Cape of South Africa, several PBNW species may occur sympatrically (Magagula 2019). Some of these, like *Sciobius tottus* Sparrman, 1785 (Entiminae: Otiorhynchinae), are consistently found in the cultivated habitat, but only occasionally populations rise above the economic threshold, decreasing again in subsequent seasons (Pringle et al. 2015, Magagula 2019). A potential threat is posed by the polyphagous nature of PBNW's, as some may host shift onto crops that are novel and/or commercially expanding in South Africa to become major pests, such as on blueberries (Bredendhand et al. 2010, Barnes et al. 2015).

Several PBNW species have successfully spread and established outside their native range; where they may become important pests. To current knowledge, three South African native species have become pests overseas. *Phlyctinus callosus* is a major introduced pest on vegetable and orchard/vineyard crops in New Zealand, Tasmania and Western

Australia (Miller 1979, Butcher 1984, Sproul et al. 1986, Fisher and Learmonth 2003, Haran et al. 2020). *Sciobius tottus* has been recorded attacking *Pinus pinaster* Aiton, 1789, on St Helena Island (Schoeman 1983), and *Afrophloeus squamifer* Boheman in Schoenherr, 1843 (Entiminae: Embrithini) canola, medics and vetch in Southern Australia (Borovec and Oberprieler 2013). A species of the genus *Systates* Gerstaecker (Entiminae: Peritilini), a South African native, has recently been collected on an ornamental plant on Reunion Island, but has not been recorded as a pest in either its native or introduced range, and the genus' host range is unknown (J.Haran unpublished data). On the receiving end; the highly polyphagous South American Naupactini species *Naupactus leucoloma* Boheman in Schoenherr, 1840 (white-fringed weevil) and *Pantomorus cervinus* Schoenherr, 1840 (Fuller's Rose Weevil), and the *Medicago* Linnaeus feeding South-Western European *Sitona discoideus* Gyllenhal, 1834 (Entiminae:Sitonini), have established successfully on their agricultural host plants in South Africa (Prinsloo and Uys 2015). Within South Africa itself, recent range expansions are observed between the south western (Mediterranean) and North Eastern (subtropical) climatic regions (*Systates* sp., *Sciobius* spp. Schoenherr, 1823, pers. obs.). As a result, South African crops may contain complex assemblages of species of PBNW for which species identification is challenging for non-specialists and an issue compounded by the presence of cryptic species in some taxa (Haran et al. 2020).

Barcode sequences of the mitochondrial gene cytochrome c oxidase I (COI) has been shown to be an accurate and powerful tool in species delimitation of most animals, including insects (Hebert et al. 2003a, Hebert et al. 2003b). Identification of pest insect taxa through barcoding has shown great potential; as it is fast, robust, accurate tool that requires relatively little tissue, may identify any life stage, and has the potential to differentiate similar species that otherwise need a high degree of taxonomic expertise to identify (Armstrong and Ball 2005, Floyd et al. 2010, Germain et al. 2013, Sow et al. 2018).

This study initiate a curated barcode database of PBNW found in crops and disturbed habitats in South Africa to assist in rapid and robust identification of species irrespective of sex or life stages of specimens.

## Material and methods

### Sampling

Specimens were collected live from agro-ecosystems with recorded damage of weevils, disturbed roadside habitat, and natural environments in South Africa (with focus on the Western Cape), between 2017 and 2019 (Suppl. material 1). One specimen of the South African native genus *Systates* was obtained from Reunion Island, and included in the study. The collection method consisted of beating/sweep netting vegetation at night/early morning, and visual searching at the base and debris on soil at base of likely host plants during the day. All specimens were stored at ambient temperature in 96% ethanol until

mounting and sequencing. Latitude and longitude were recorded, as well as host plant record where possible.

Species were identified based on external morphology using the keys and descriptions of Schoeman 1983, Oberprieler 1988, Borovec and Oberprieler 2013, Prinsloo and Uys 2015 and Haran et al. 2020. Reference collections housed at Iziko museum (Cape Town, South Africa) and South African National Collection of Insects (SANCO, Pretoria) were also consulted to cross validate identifications with type material and specimens identified by specialists (Suppl. material 1). The male external genitalia have high diagnostic value in Entiminae (Borovec and Skuhrovec 2018, Borovec 2019, Haran et al. 2020). For genera requiring the dissection of internal genital structures, the full abdomens were extracted, and soft tissue digested with KOH to obtain external genitalia (penis, copulatory sclerite and tegmen of males, and sternite VIII, spermatheca and gonocoxites of females). All vouchers specimens (including their genitalia stored in glycerol) were mounted on a card and deposited in the collections listed in Suppl. material 1. Two cyclomine weevil species collected in disturbed agricultural habitats are included in this study; a native *Rhyparosomus* sp. Schoenherr, 1842, (Cyclominae: Rhythirrinini), and the exotic vegetable weevil, *Listroderes costirostris* Schoenherr, 1826, (Cyclominae: Rhythirrinini), which is a common pest on vegetables in the Western Cape Province of South Africa (Visser 2009). All of the weevil species in this study, apart from *N. leucoloma*, *P. cervinus*, *S. discoideus* and *L. costirostris* are native to South Africa. When possible, multiple specimens per species were sequenced in order to estimate the level of intraspecific distances encountered in these taxa (Suppl. material 1).

## DNA extraction, amplification and sequencing

The right back leg of each prepared specimen was used for DNA extraction. The DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). PCR amplification was done for the *COI* standard barcoding region (Hebert et al. 2003b) of invertebrates using standard primers (traditional Folmer et al. 1994), that have been adapted by Germain et al. 2013 to a primer cocktail, and M13-tails added, to increase amplification success and allow for sequencing respectively (Ivanova et al. 2007), Table 1. PCR reactions were carried out in a Mastercycler Nexus (Eppendorf, Hamburg, Germany) with a final volume of 10  $\mu$ L containing 5  $\mu$ L of Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 2  $\mu$ M of each primer and 2  $\mu$ L of DNA template. The PCR conditions were as follows: initial DNA denaturation at 94°C for 15 min, followed by 35 cycles of 30s at 94°C, 1 min at 52°C and 1 min at 72°C, and a final extension of 15 min at 72°C. The PCR products were paired-end sequenced by Eurofins Genomics (<http://www.eurofinsgenomics.eu/>).

## Sequence analysis

The barcode sequences were aligned and manually checked using CodonCode Aligner ver. 3.7.1 (CodonCode Corporation, Centerville, MA, USA), verifying the absence of pseudogenes using standard detection methods (Haran et al. 2015). The sequences and

GenBank codes obtained for multiple specimens of the six *Phlyctinus* species in Haran et al. 2020 are reported again in this study (Suppl. material 1, NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>, codes MN627231-MN627250) and used as data in further sequence analysis. Pairwise sequence divergences were calculated using the Kimura-2-Parameter, K2P, (Kimura 1980) in MEGA7 (Kumar et al. 2016), utilizing the 'pairwise-deletion of gaps' option (Suppl. material 2). Pairwise sequence divergence was visualized on a Neighbourhood Joining (NJ) tree (Saitou and Nei 1987) using K2P distances in MEGA7 (Fig. 1). All specimens that showed a intraspecific K2P distances of  $\geq 2\%$  from their conspecifics are given a distinct haplotype ('H') differentiation (Suppl. material 1). This threshold was decided upon based on the deep intraspecific variation shown by most species with multiple sequences obtained, thresholds used by other studies on Lepidoptera and Coleoptera (Bergsten et al. 2012, Mutanen et al. 2012) and the flightless nature of most PBNW species.

For all barcoded specimens the species identification, collection data, images, primer cocktails used in amplification, sequences, trace files and voucher data were uploaded to the Barcode of Life Database (BOLD; <http://www.boldsystems.org/>) under the project name CURSA [http://www.boldsystems.org/userfiles/CURSA/specimen\\_data\\_file\\_CURAF%2B1607592215.xls](http://www.boldsystems.org/userfiles/CURSA/specimen_data_file_CURAF%2B1607592215.xls) (Hansen et al. 2020), and subsequently deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>, codes MT674814-MT674861), Suppl. material 1.

## Data resources

The collection and voucher data of all specimens used in this study is deposited at BOLD (Ratnasingham and Hebert 2007), the Barcode of Life Data System, under project name CURSA, [http://www.boldsystems.org/userfiles/CURSA/specimen\\_data\\_file\\_CURAF%2B1607592215.xls](http://www.boldsystems.org/userfiles/CURSA/specimen_data_file_CURAF%2B1607592215.xls), (Hansen et al. 2020).

The COI sequence data of the *Phlyctinus* spp. (JHAR0941-0101, JHAR0755-0101, JHAR2288-0101, JHAR2290-0101, JHAR2086-0101, JHAR2101-0101, JHAR0822-0101, JHAR1353-0101, JHAR2252-0101, JHAR2252-0101, JHAR0819-0101, JHAR1016-0101, JHAR2173-0101, JHAR1304-0101, JHAR1195-0101, JHAR0973-0101, JHAR1300-0101, JHAR1082-0101, JHAR2264-0101) and *Oosomus* sp. (JHAR01073-0101) obtained in Haran et al. 2020 are deposited at NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), codes MN627231-MN627250 (Suppl. material 1).

The COI sequence data obtained and unpublished by Magagula 2019 in a thesis at Stellenbosch University, and sequences originally obtained in the current study are deposited at NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), codes MT674814-MT674861 (Suppl. material 1).

## Results

A total of 70 COI barcode sequences >500 bp, from 41 morphospecies (39 Entiminae, 2 Cyclominae), are presented in this study. Of these, all have been identified to genus level; 29 to species level, and 12 of the morphospecies was either not possible to identify, or can't be identified with certainty (Suppl. material 1). No evidence was found of pseudogene amplification, and no sequences were shared between species. A total of 50 new sequences from 35 morphospecies, 23 of which have been identified with certainty to species level, have been deposited in GenBank (NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), codes MT674814-MT674861, Suppl. material 1). This includes previously unpublished sequences of PBNW's from vineyards and apple orchards in the Western Cape of South Africa, obtained by Magagula 2019 in a thesis. Only three specimens did not amplify (Suppl. material 1), one of which was a dried museum specimen. All the COI sequences except MN627242 (*Phlyctinus xerophilus* Haran, 2020, 615 bp), MN627249 (*Phlyctinus grootbosensis* Haran, 2020, 563 bp), and MT674827 (*Eremnus laticeps* Boheman, 1843, 649 bp) are 658 bp in length, and include the 648 bp barcoding region for animals (Hebert et al. 2003b).

Of the 14 species for which more than one sequence could be obtained, eight had K2P intraspecific variation  $\geq 1\%$ . Of these, 5 had intraspecific K2P variation of 2% or higher, and were divided into haplotypes (Suppl. material 1). These species and their maximum intraspecific K2P distance obtained in this study are: *Afrophloeus spathulatus* Boheman in Schoenherr, 1843 (6.1%), *Eremnus atratus* Sparrman, 1785 (2.8%), *Eremnus horticola* Marshall, 1920 (2.0%), *P. callosus* (3.0%), *P. xerophilus* (9.2%). The specimens of *Systates* sp. (MT674859, MT674860), *Sciobius pollinosus* Fahraeus, 1871 (MT674854) and *Sciobius cf. pollinosus* (MT674853) (Suppl. material 1) are considered separate species in subsequent analysis, due to the specimens not being able to be identified morphologically with certainty, and the K2P distance between the specimens of 11.2% and 9.7% respectively.

Among the present dataset of barcode sequences, the mean intraspecific distance is 2.1% (max 9.2%, min 0.0%), the mean of the maximum intraspecific distances is 2.2%, and the mean distance to the nearest heterospecific is 13.9%. The smallest interspecific distance is 4.3%. There some overlap between intra-and-interspecific distances, and a small barcoding gap (mean distance to nearest heterospecific being 6.3 fold higher than the mean of maximum intraspecific distances) with this conservative method of calculation. Out of the 14 species for which two or more sequences could be obtained, six had maximum intraspecific distances of  $\leq 10\%$  that of distance to closest heterospecific, and 10 had intraspecific distances  $\leq 12\%$  that of distance closest to heterospecific. Except for *P. xerophilus*, the distance to the nearest heterospecific was always larger than the maximum intraspecific value.

The NJ tree of the obtained sequences show distinct, non-monophyletic groupings between species in the genera *Eremnus* Schoenherr, 1826, *Afroleptops* Oberprieler,

1988, and *Sciobius*; and non-monophyletic groupings between genera and/or species in the tribes Tanyrhynchini (genera *Eremnus*, *Tanyrhynchus* Schoenherr, 1826, and *Afroleptops* in this study), Embrithini (genera *Afrophloeus* Borovec and Oberprieler, 2013, and *Ellimenistes* Boheman, 1843, in this study), and Otorhynchinae (genus *Sciobius* in this study) (Fig. 1).

## Discussion

Barcoding PBNW species from South Africa provide a valuable tool in rapidly and robustly identifying species of potential economic concern, including the highly sporadic pest species. This study also reveal that some challenges exist with the application of this approach to PBNW in South Africa. A small barcoding gap between mean of maximum intraspecific genetic distance and mean distance to closest heterospecific is observed, even without comprehensive sampling and multiple sequences per species for most of the PBNW's in this study. However, this result is not unexpected for Coleoptera (Meier et al. 2008, Bergsten et al. 2012), or indeed in other groups that have been well sampled (Meyer and Paulay 2005). The significant geographic distance between many of the sampled specimens in taxa where multiple sequences were obtained probably contributes to the high intraspecific genetic distances here observed (Bergsten et al. 2012). This need however, not be impediment to correct species identification using barcodes, provided adequate sampling and correct taxonomy of the group in question (Ross et al. 2008). Deep intraspecific variation (often higher than 2%) and a ratio of largest intraspecific distance to nearest heterospecific distance often higher than 10% observed in this study will decrease the accuracy of identification (Ross et al. 2008). However, we believe the decrease in identification accuracy to be low enough to still allow for practical use, especially if taxonomy in historically challenging groups like *Eremnus* and *Phlyctinus* (Oberprieler 1988, Haran et al. 2020) becomes better resolved.

The largest challenge to successful barcoding of PBNW is undoubtedly inadequate taxonomic coverage of this group. The taxonomy of South African Entiminae is complex and many tribal/genus/species classification is still in the process of being resolved (Oberprieler 1988, Borovec and Oberprieler 2013, Haran et al. 2020). Some taxa consist of complexes of cryptic species that need a high degree of taxonomic expertise to identify (Haran et al. 2020), and are thus easily 'overlumped' (Funk and Omland 2003) by taxonomists. The dangers of inadequate taxonomy to a barcoding initiative are well illustrated by the genus *Phlyctinus*; which were long treated as a single species, *P. callosus*. Recent taxonomic work has split this taxon into six close related species (Haran et al. 2020). Even now the newly named species *P. xerophilus* may itself constitute a complex of cryptic species (Haran et al. 2020, Suppl. material 1); if this proves to be the case, the barcoding gap observed in this study would increase and the overlap between intra-and-interspecific distances would decrease. In the present study, about 36% of the species for which more than 1 sequence could be obtained showed substantial genetic divergence between intra specific lineages. Although the sequencing of a complementary nuclear gene

is required to determine if these lineages are reproductively isolated, such ratio gives a first indication on priority for futures taxonomic treatments of pest species.

The NJ tree calculated using the COI sequences itself provides some preliminary evidence that some South African genera and tribes previously described only on morphological characteristics do not form naturally monophyletic groupings (Fig. 1). It was already noted by Oberprieler 1995 and Borovec and Oberprieler 2013 that the tribes Embrithini and Tanyrhynchinae, and the genus *Eremnus* within Tanyrhynchinae (Oberprieler 1988), may not form natural monophyletic groupings and are in need of comprehensive taxonomic revision and phylogenetic studies. It is surprising that all members of the genera *Sciobius* and *Afroleptops* sequenced did not group together in the NJ tree (Fig. 1), as these genera were considered monophyletic based on morphological characteristics (Schoeman 1983, Oberprieler 1988). Although conclusions should not be drawn from these results on a single gene region; further taxonomic and phylogenetic investigation into these taxa are warranted.

The third challenge is insufficient sampling to adequately cover genetic diversity across the target groups distribution range (Bergsten et al. 2012); especially within biodiversity hotspots for PBNW's such as occur in the Western Cape (Oberprieler 1988, Haran et al. 2020). This may be compounded by the poor natural dispersal ability of typically flightless PBNW's, potentially allowing for relatively high genetic subdivision of different populations of the same species (Peterson and Denno 1997). This may potentially explain what is being observed in the *Systates* sp.'s from RSA and Reunion Island, and *P. callosus* from different areas within what is considered its native distribution, in this study.

A curated barcoding database (as on BOLD and GenBank) will enable the addition of sequences and species with every new study, allowing for taxonomic amendment. It can include location data, such that the robustness and accuracy in identifying specimens of the group of interest can continuously be improved (Armstrong and Ball 2005, Floyd et al. 2010).

The major advantages of the presented curated barcodes for PBNW's is that it allows for the identification by non-specialists of taxa that otherwise require a high degree of taxonomic expertise to identify (Floyd et al. 2010, Germain et al. 2013). The second major advantage is any life stage and sex may be identified by barcoding with the same level of certainty as adults (Floyd et al. 2010, Germain et al. 2013). In some taxa such as *Phlyctinus*, females do not have robust external features that allow for species differentiation (Haran et al. 2020). The life history of many PBNW species still remain a mystery; this database will allow identification of eggs, larvae and pupae that might, as such, be linked to a specific host plant and/or soil type. The published COI barcodes of key PBNW species, together with properly photographed and curated voucher specimens of species placed in a museum, allow for a greater scope of study on the taxonomy, biology, distribution and host range of potential and recognized PBNW pests (Floyd et al. 2010, Germain et al. 2013).

## Conclusions

The ability to accurately identify agricultural pest insects is key in their successful management. The standard barcoding gene, COI, is a useful tool in modern insect pest identifications, as it can be used to identify any life stage, uses relatively little tissue, the methods for DNA extraction and sequencing are standardized, and reference sequences linked to voucher data, images, primer cocktails and trace files are openly available on GenBank and BOLD; making it possible for non-experts to identify an unknown insect for which these data exist. Furthermore, these data can continually be expanded and improved as more species and specimens are sequenced, and as taxonomical work progresses and improves current classifications; increasing the diagnostic power of barcode identifications over time.

The PBNWs of South Africa and are a diverse and taxonomically understudied group that contains a number of important pest species-and strong potential for future pest emergence due to their polyphagous nature. Their identification provides a challenge for non-specialists; an issue compounded by the presence of cryptic species in some taxa. A curated barcode database provides a quick and simple identification tool that allows for a better understanding of their taxonomy, biology, distribution.

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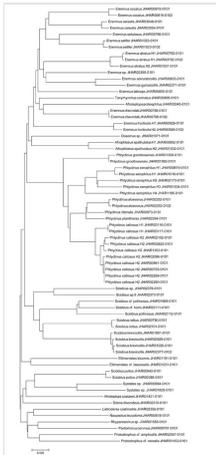


Figure 1.

Neighbour-Joining tree of the COI sequences for polyphagous broad-nosed weevils obtained/used in study.

Table 1.

PCR primers and conditions. M13 tails from Ivanova et al. (2007) are in bold.

Gene	Primer	Primer Sequence	Annealing temperature	Reference
COI	HCO2198	<b>CAGGAAACAGCTATGACT</b> AAACYTCGGATGBCCAAARAATCA	52°C	Folmer et al. (1994), modified in Germain et al. (2013)
		<b>CAGGAAACAGCTATGACT</b> AAACYTCAGGATGACCAAAAAYCA		
		<b>CAGGAAACAGCTATGACT</b> AAACTTCWGGRTGWCCAAARAATCA		
	LCO1490	<b>TGTAAACGACGGCCAGT</b> TTTCAACTAAYCATAARGATATYGG		
		<b>TGTAAACGACGGCCAGT</b> TTTCAACWAATCATAAAGATATTGG		

## Supplementary materials

### Suppl. material 1: Supplementary 1. BDJ\_15510

**Authors:** Steffan Hansen, Pia Addison, Laure Benoit and Julien Haran

**Data type:** GenBank accession codes and collection data

**Brief description:** Voucher specimen codes, GenBank accession codes (where applicable) for COI sequences, and collection data of the polyphagous broad-nosed weevils in the study

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### Suppl. material 2: Supplementary 2. BDJ\_15510

**Authors:** Steffan Hansen, Pia Addison, Laure Benoit and Julien Haran

**Data type:** K2P pairwise genetic distances

**Brief description:** The Kimura-2-Parameter pairwise genetic distances between COI sequences for polyphagous broad-nosed weevils obtained/used in study

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