Identification of Central European ground beetles of the genus *Bembidion* (Coleoptera: Carabidae) using DNA barcodes: A case study of selected species[1]

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[1] Dedicated to Prof. Gerd Müller-Motzfeld (†)

Abstract: Identification of Central European ground beetles of the genus *Bembidion* (Coleoptera: Carabidae) using DNA barcodes: A case study of selected species - Species identification based on morphological characters is challenging when closely related species are found at a given locality. In this context, the identification of immature stages is difficult, especially due to lack of distinct morphological differences. Sequence diversity within a fragment of the mitochondrial cytochrome *c* oxidase subunit 1 gene (COI), the so-called DNA barcode for animals, has been shown to represent an effective molecular tool for valid species identification. In this study we examined the performance of DNA barcodes in the discrimination of 21 ground beetle species of the genus *Bembidion* from Central Europe, analysing 101 specimens. Our results revealed low intraspecific variation (mean = 0.19%) and a clear barcoding gap. Although further work is needed to examine patterns of sequence diversity on a broader geographical scale as well as more specimens and additional species, our case study encourages the use and effectiveness of DNA barcodes for a reliable identification of Central European *Bembidion* species.

1 Introduction

The Carabidae count as one of the largest and most diverse insect families, with no less than an estimated 40,000 described species inhabiting all terrestrial habitat types from the sub-arctic to wet tropical regions (Arnett & Thomas 2000, Arndt et al. 2005). Ground beetles, which constitute more than 750 species, are also an important element of the Coleopteran fauna of Central Europe (Müller-Motzfeld 2004). Carabidae are known to be a species-rich group with a high level of morphological and ecological diversity (e.g. Lövei & Sunderland 1996), exhibiting different levels of habitat selectivity that range from generalist to specialist. As a consequence, carabids are often numerically dominant in collections of ground-dwelling arthropods and can be used as indicator organisms for assessments of environmental pollution, habitat classification, conservation, or the characterization of soil nutrients (e.g. Lövei & Sunderland 1996, Rainio & Niemelä 2003, Avgin & Luff 2010). However, many species are difficult to identify morphologically and usually require the help of highly trained taxonomists due to different degrees of morphological variability and polymorphisms within and between species. It is also very difficult or impossible to identify the larvae or females of some species, e.g. various *Amara* species (Hurka 1996).

In this context, DNA barcodes may represent a new effective tool for valid species identification for large-scale biodiversity studies (e.g. Valentini et al. 2008, Smith & Fisher 2009, Hebert et al. 2010). During recent years DNA barcoding has emerged as a rapid method for species discovery and biodiversity assessment (e.g. Hajibabaei et al. 2006, Stoeckle & Hebert 2008, Radulovici et al. 2009, Zhou et al. 2009). A DNA barcode itself consists of a 648 bp region of the mitochondrial cytochrome *c* oxidase 1 (COI) gene. The concept of DNA barcoding is based on the assumption that each species will most likely have a unique DNA barcode and that intraspecific COI variation is lower than interspecific COI variation, generating the so-called barcoding gap (Hebert

et al. 2003a, 2003b, 2004). In contrast to DNA taxonomy, which focuses on the classification of both known and undescribed species (Tautz et al. 2003, Vogler & Monagham 2007), the main goal of DNA barcoding is to identify unknown specimens in terms of a known and existing classification (Hebert et al. 2003a). The effectiveness of DNA barcoding for the identification of species has been proven in many studies, analysing both vertebrate and invertebrate taxa (e.g. Clare et al. 2007, Costa et al. 2007, Hubert et al. 2008, Machida et al. 2009, Rivera & Currie 2009, Robinson et al. 2009, Uthicke et al. 2010). However, there are only few studies that analyse the usefulness of COI barcodes for the species identification of ground beetles (Greenstone et al. 2005, Maddison 2008, Raupach et al. 2010a, 2010b).

In this study we tested the ability of DNA barcodes to discriminate a variety of Central European species of the genus *Bembidion*. The genus *Bembidion* is the largest genus within the Carabidae with more than 1,200 species worldwide (Lindroth 1985, Maddison 2008). All species are small (less than 9 mm), move very fast and most species live close to water (Lindroth 1985). We analysed a subset of 21 species, including a variety of morphologically similar and obviously closely related or sibling species, such as the species pairs e.g. *Bembidion lampros/Bembidion properans* (Desender & Crappe 1983, Müller 1971), *Bembidion millerianum/Bembidion ruficorne* (Reitter 1908, Müller-Motzfeld 1982) or *Bembidion deletum/Bembidion incognitum (*Müller-Motzfeld 2004).

2 Material and Methods

In total, 101 adult ground beetle specimens of the genus *Bembidion* were collected by the authors in various localities in Austria and Germany (Tab. 1). All beetles were identified by one of the authors of this article (KH) using the keys in Müller-Motzfeld (2004). Numbers of analysed specimens per species ranged between two and ten beetles. Specimens are deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn, Germany. Total genomic DNA was extracted from dissected legs of specimens or complete specimens using the QIAmp© Tissue Kit (Qiagen GmbH, Hilden), following the manufacturer's extraction protocol. Polymerase chain reaction (PCR) has been used for amplifying the COI barcode fragment using the primer pair LCO1480 and HCO2198 (Folmer et al. 1994). The PCR mix contained 4 µl Q-Solution, 2 µl 10x Qiagen PCR buffer, 2 µl dinucleotide triphosphates (dNTPs, 2 mmol/µl), 0.1 µl of each primer (both 50 pmol/µl), 1 µl of DNA template with of between 2 and 150 ng/µl, 0.2 µl Qiagen Taq polymerase (5 U/µl), and filled up to 20 µl with sterile H_2O. PCR amplification reactions were conducted in Thermal Cycler GeneAmp© PCR System 2700/2720 (Applied Biosystems, Darmstadt) or the Eppendorf Mastercycler© Pro system. The PCR thermal conditions included an initial denaturation at 94 °C (5 min), followed by 38 cycles at 94 °C (denaturation, 45 s), 48 °C (annealing, 45 s), 72 °C (extension, 80 s), and a final extension step at 72 °C (7 min). Negative and positive controls were included with each round of reactions. All PCR products were purified with the QIAquick© PCR Purification Kit (Qiagen GmbH, Hilden). Purified PCR products were cycle-sequenced and sequenced in both directions at a contract sequencing facility (Macrogen, Seoul, Korea) on an ABI3730 XL automatic DNA sequencer, using the same primers as used in PCR. Double-stranded sequences were assembled using the SeqMan™ II programme (DNASTAR, Inc., Konstanz, Germany) or the Geneious© 5.3 programme package (Drummond et al. 2010). We also performed BLAST searches to confirm the identity of all new COI sequences (Altschul et al. 1990). Aligned sequences were translated to amino acid sequences to check for nuclear mitochondrial pseudogenes (numts) using Geneious© 5.3 (Drummond et al. 2010). Various *Bembidion* COI sequences were already published as part of a comprehensive study analysing the utility of DNA barcodes and nuclear rDNA expansion segments for carabid species identification (see Raupach et al. 2010a). All analysed sequences are available in GenBank (see Tab. 1).

Sequences were aligned using MUSCLE version 3.6 (Edgar 2004) with default settings. The analysis of intra- and interspecific genetic variability of the analysed *Bembidion* species based on Kimura 2-parameter distances (K2P; Kimura 1980) were calculated using MEGA 5.03 (Tamura et al. 2011). Intra- as well as interspecific K2P distances were plotted as histogram (Figure 1) using PAST version 1.94b (Hammer et al. 2001). Base frequencies were obtained using MEGA 5.03 (Tamura et al. 2011). We also performed a neighbour-joining cluster analysis (Saitou & Nei 1987) for a graphical repre-

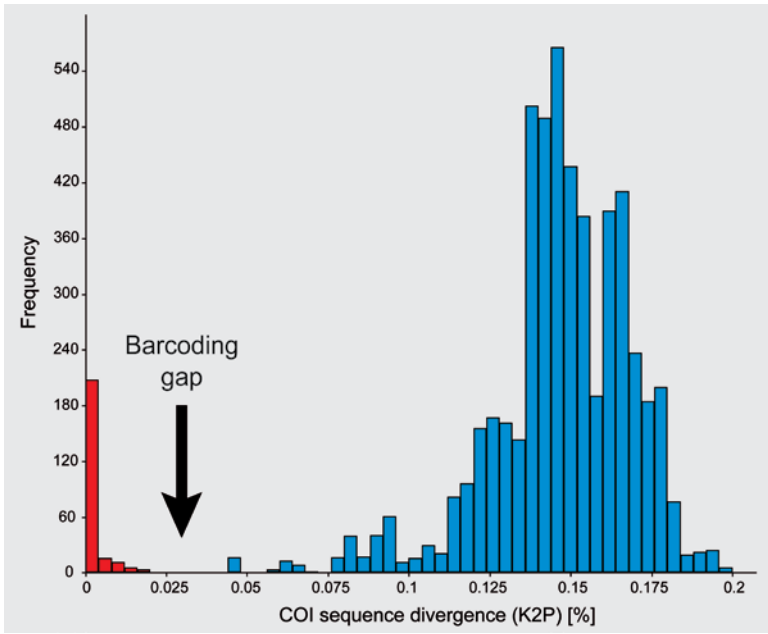

Fig. 1: Histogram of the calculated intra- and interspecific Kimura 2-parameter distances of the COI barcode fragment for the analysed *Bembidion* specimens.

sentation of patterns of nucleotide divergences using MEGA 5.03, based on K2P distances (Figure 2). Four COI sequences of *Omophron limbatum* were used as outgroup (GenBank accession numbers GU347265-GU347268). Finally, bootstrap support values were calculated by re-sampling and analysing 1,000 replicates (Felsenstein 1985).

3 Results

Our analysis revealed low GC-contents (32.6 %) for the analysed sequences, as known in most insects (Min & Hickey 2007, Clare et al. 2008, Wei et al. 2010). Individual mean nucleotide content was A = 0.299, G = 0.166, C = 0.16 and T = 0.375. The analysis of pairwise COI nucleotide divergences for all *Bembidion* species showed higher interspecific than intraspecific divergences (Figure 2). All specimens of the same species grouped together, even when samples where obtained from geographically disparate areas. Consequently, all analysed species were identified in the sampled material and a clear barcoding gap was revealed. Mean intraspecific genetic divergence for the analysed 21 *Bembidion* species was 0.19 %. Maximum intraspecific divergence was observed in *Bembidion properans* (1.92 %), followed by *Bembidion testaceum* (1.27 %) and *Bembidion litorale* (0.95 %). Interspecific distances ranged between 0.0458 and 0.1998 (mean = 14.71 %). Lowest interspecific distances were found between *Bembidion millerianum* and *Bembidion ruficorne* (4.58 – 4.75 %), indicating a close relationship between both species.

It is very important to mention that DNA barcoding focuses on species delineation and identification, and not on phylogenetic inference. Therefore, the presented topology (Figure 2) does not show reliable phylogenetic relationships between the analysed taxa.

4 Discussion

When it began in 2003, the International Barcode of Life project (www.ibol.org) was conceived as a standard system for a fast, valid and accurate identification of animal species (Hebert et al. 2003a, Miller 2007). In this context, DNA barcodes help to assign unidentified specimens to known species as well as to identify new species, especially taxa that have a complex or inaccessible morphology (Hebert et al. 2003a). One obvious advantage of DNA barcoding comes from the rapid and standardized acquisition of molecular data, using conventional and inexpensive protocols for DNA extraction, amplification and sequencing (Borisenko et al. 2009, Ivanova et al. 2009). Most importantly, it facilitates the compilation of a precise, representative open-access reference library (BOLD, www.boldsystems.org). To

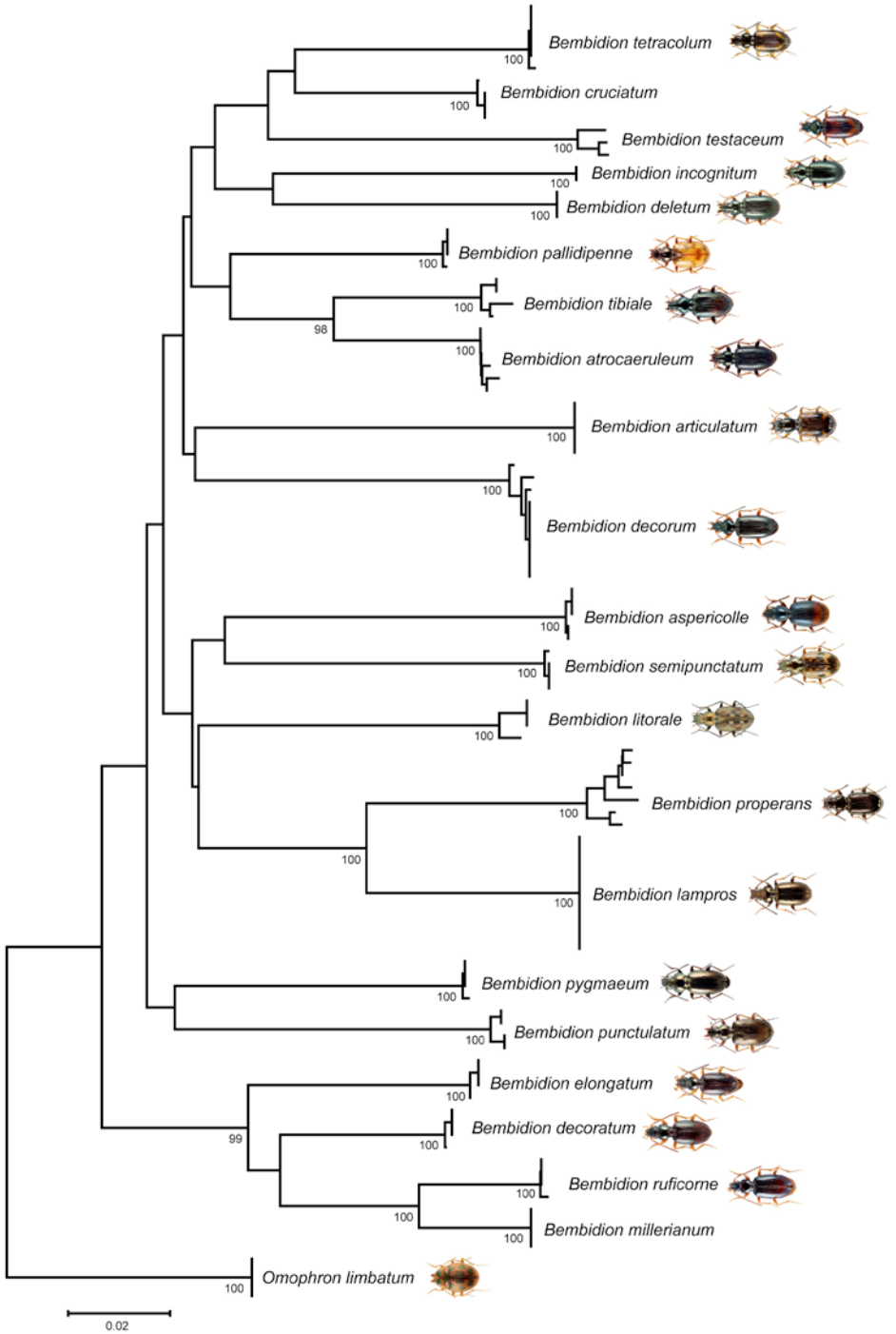

Fig. 2: Neighbour-joining topology based on Kimura 2-parameter distances as well as images of selected analysed species. Numbers next to internal branches are bootstrap values [%], which are only given if they had a value of 90 or more.

date, (24.07.2011), 1,312,754 barcodes of 107,794 different species have been published. In contrast to other traditional sequence data libraries (e.g. NCBI or EMBL), BOLD is also an interactive interface where deposited sequences can be revised and taxonomically reassigned (Ratnasingham & Hebert 2007).

Despite the fact that DNA barcoding is still subject to criticism, viewed with reservation and/or simply not understood (e.g. DeSalle et al. 2005, Will et al. 2005, Klausnitzer 2010, Ebach 2011), barcoding has become an established and highly efficient method for understanding and assessing biodiversity (Valentini et al. 2008, Jinbo et al. 2011). All around the world, numerous projects have been started to compile reference libraries from specimens of various taxa whose identity is already firmly established (e.g. Smith & Fisher 2009, Bucklin et al. 2010, Hausmann et al. 2011, Lakra et al. 2011). Besides this, various national and regional campaigns, for example NorBoL (Norway, www.dnabarcoding.no), JBoL (Japan, www.jboli.org), or Barcoding Fauna Bavaria (www.faunabavarica.de), as well as global taxa-specific campaigns (e.g. relating to Porifera (www.spongebarcoding.org), fishes (www.fishbol.org), birds (www.barcodingbirds.org), ants (www.formicidaebol.org) or butterflies (www.lepbarcoding.org) have started to support the iBoL initiative. Initial results show that in many cases there are more species – each more narrowly specialized – than scientists had realized before, e.g. within butterflies (Hebert et al. 2004, Strutzenberger et al. 2011), molluscs (Johnson et al. 2008), amphipods (Costa et al. 2010), and fishes (Zemlak et al. 2009). A preliminary study of 75 Central European ground beetles revealed the existence of cryptic species within *Nebria hellwigii* (Raupach et al. 2010a). However, the use of mitochondrial gene fragments for species identification is not without problems: pseudogenes (numts) (e.g. Bensasson et al. 2000, Kim et al. 2006, Koutroumpa et al. 2009), introgressive hybridization (e.g. Sota et al. 2001, Croucher et al. 2004, Zhang & Sota 2007), or maternally inherited α-proteobacteriae (e.g. *Wolbachia*) (Duron et al. 2008, Werren et al. 2008) can influence mitochondrial gene variability. In such cases, the analysis of supplementary nuclear markers is necessary (Raupach et al. 2010a).

Besides a valid and accurate identification of animal species, DNA barcodes can also support other scientific domains, for example ecology (Valentini et al. 2008), forensics (Dawnay et al. 2007, Nelson et al. 2007, Ferri et al. 2009, Meiklejohn et al. 2011), pest biology (Engstrand et al. 2010), evolutionary biology (e.g. Clare et al. 2008, da Silva et al. 2011), invasive species biology (Armstrong & Ball 2005, Darling & Blum 2007), biogeography (e.g. Linares et al. 2009), and conservation biology (Ward et al. 2008, Neigel et al. 2008). New insights into ecology and species biology have already emerged from various DNA barcode studies (Frézal & Leblois 2008). For example, the identification of organisms found in stomach extracts allows the elucidation of wild animal diets, especially when behavioural studies are not feasible (see Valentini et al. 2008). In this context, newly emerging sequencing techniques such as pyrosequencing (454 Roche, Solexa, SOLID) enable rapid and representative analyses of mixed samples (e.g. stomach contents, food, blood or water columns) (Valentini et al. 2008). Moreover, the emerging field of metagenomics will also support future DNA barcoding initiatives (Hudson 2008).

Our study confirms the usefulness of DNA barcodes for the identification of species of the genus *Bembidion*. We found a clear barcoding gap that allows a firm identification of all analysed species, including closely related species pairs such as *Bembidion lampros/properans* and *Bembidion millerianum/ruficorne* (see Figure 2). We also gained some first impressions of the intraspecific variability of various *Bembidion* species. Of course, it is clear that additional specimens and species have to be analysed to evaluate intra- and interspecific COI variability in more detail in the near future. To summarize the results, our study confirms the high potential of DNA barcodes for a successful species identification of even closely related ground beetle species of the genus *Bembidion* in Central Europe.

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Tab. 1: Species identification, voucher code, location and year of collection as well as GenBank accession numbers of the 101 analysed *Bembidion* specimens. Country codes: GER (Germany): NW (North-Rhine Westphalia), NI (Lower Saxony), SH (Schleswig–Holstein), RP (Rhineland-Palatinate), ST (Saxony–Anhalt); AU (Austria): K (Carinthia).

Species	ZFMK voucher code	Locality	COI accession number
Bembidion articulatum (Panzer, 1796)	ZFMK_COL_2008_44	Bienen, NW, GER, 2006	GU347065
Bembidion articulatum (Panzer, 1796)	ZFMK_COL_2008_99	Waltrop, NW, GER, 1999	GU347068
Bembidion articulatum (Panzer, 1796)	ZFMK_COL_2008_119	Waltrop, NW, GER, 1999	GU347069
Bembidion articulatum (Panzer, 1796)	ZFMK_COL_2008_159	Waltrop, NW, GER, 1999	GU347067
Bembidion articulatum (Panzer, 1796)	ZFMK_COL_2009_38	Schmedehausen, NW, GER, 2008	GU347066
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2009_350	Meindorf, NW, GER, 2008	JF895158
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2009_351	Meindorf, NW, GER, 2008	JF895159
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2009_352	Neheim, NW, GER, 2008	JF895160
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2011_292	Arnsberg, NW, GER, 2008	JF895155
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2011_293	Arnsberg, NW, GER, 2008	JF895156
Bembidion atrocaeruleum (Stephens, 1828)	ZFMK_COL_2011_294	Arnsberg, NW, GER, 2008	JF895157
Bembidion aspericolle (Panzer, 1796)	ZFMK_COL_2008_23	Teutschenthal, ST, GER, 2005	GU347070
Bembidion aspericolle (Panzer, 1796)	ZFMK_COL_2008_24	Teutschenthal, ST, GER, 2005	GU347072
Bembidion aspericolle (Panzer, 1796)	ZFMK_COL_2008_25	Teutschenthal, ST, GER, 2005	GU347073
Bembidion aspericolle (Panzer, 1796)	ZFMK_COL_2008_26	Teutschenthal, ST, GER, 2005	GU347074
Bembidion aspericolle (Panzer, 1796)	ZFMK_COL_2008_27	Teutschenthal, ST, GER, 2005	GU347071
Bembidion cruciatum Dejean, 1831	ZFMK_COL_2009_355	Feistritz, K, AU, 2008	JF895169
Bembidion cruciatum Dejean, 1831	ZFMK_COL_2009_356	Feistritz, K, AU, 2008	JF895170
Bembidion cruciatum Dejean, 1831	ZFMK_COL_2009_357	Feistritz, K, AU, 2008	JF895171
Bembidion cruciatum Dejean, 1831	ZFMK_COL_2009_358	Maria Elend, K, AU, 2008	JF895172
Bembidion decoratum (Duftschmid, 1812)	ZFMK_COL_2009_361	Maria Elend, K, AU, 2008	GU347075
Bembidion decoratum (Duftschmid, 1812)	ZFMK_COL_2009_362	Maria Elend, K, AU, 2008	GU347076
Bembidion decoratum (Duftschmid, 1812)	ZFMK_COL_2009_363	Maria Elend, K, AU, 2008	GU347077
Bembidion decoratum (Duftschmid, 1812)	ZFMK_COL_2009_364	Maria Elend, K, AU, 2008	GU347078
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2008_12	Neheim, NW, GER, 2004	GU347079
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2008_13	Neheim, NW, GER, 2004	GU347081
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2008_14	Neheim, NW, GER, 2004	GU347082
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2008_16	Bienen, NW, GER, 2004	GU347083
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2008_42	St. Augustin-Meindorf, NW, GER, 2008	GU347080
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2009_360	St. Augustin-Meindorf, NW, GER, 2008	GU347085
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2009_400	St. Augustin-Meindorf, NW, GER, 2008	GU347084
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2009_498	St. Augustin-Meindorf, NW, GER, 2008	GU347086
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2009_499	St. Augustin-Meindorf, NW, GER, 2008	GU347087
Bembidion decorum (Panzer, 1799)	ZFMK_COL_2009_500	Bienen, NW, GER, 2008	GU347088
Bembidion deletum Audinet-Serville, 1821	ZFMK_COL_2009_366	Hochobir, K, AU, 2008	JF895161
Bembidion deletum Audinet-Serville, 1821	ZFMK_COL_2009_367	Hochobir, K, AU, 2008	JF895162
Bembidion deletum Audinet-Serville, 1821	ZFMK_COL_2009_368	Hochobir, K, AU, 2008	JF895163
Bembidion elongatum Dejean, 1831	ZFMK_COL_2009_40	Schmedehausen, NW, GER, 2008	GU347089
Bembidion elongatum Dejean, 1831	ZFMK_COL_2009_41	Schmedehausen, NW, GER, 2008	GU347090
Bembidion elongatum Dejean, 1831	ZFMK_COL_2009_42	Schmedehausen, NW, GER, 2008	GU347091
Bembidion elongatum Dejean, 1831	ZFMK_COL_2009_43	Schmedehausen, NW, GER, 2008	GU347092
Bembidion incognitum Müller, 1931	ZFMK_COL_2009_370	Gurktaler Alpen, K, AU, 2008	JF895164
Bembidion incognitum Müller, 1931	ZFMK_COL_2009_371	Gurktaler Alpen, K, AU, 2008	JF895165
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2008_122	Borkum, NI, GER, 2005	GU347094
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2008_142	Arnsberg, NW, GER, 2007	GU347096
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2008_146	Haltern-Lavesum, NW, GER, 1999	GU347097
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_53	Schmedehausen, NW, GER, 2008	GU347101
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_54	Schmedehausen, NW, GER, 2008	GU347102
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_611	Teutschenthal, ST, GER, 2008	GU347098
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_612	Teutschenthal, ST, GER, 2008	GU347093
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_613	Teutschenthal, ST, GER, 2008	GU347099
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_614	Teutschenthal, ST, GER, 2008	GU347100
Bembidion lampros (Herbst, 1787)	ZFMK_COL_2009_644	Wyk, SH, GER, 2003	GU347095
Bembidion litorale (Olivier, 1790)	ZFMK_COL_2009_59	Greven-Bockholt, NW, GER, 2008	GU347103
Bembidion litorale (Olivier, 1790)	ZFMK_COL_2009_60	Greven-Bockholt, NW, GER, 2008	GU347105
Bembidion litorale (Olivier, 1790)	ZFMK_COL_2009_61	Greven-Bockholt, NW, GER, 2008	GU347106
Bembidion litorale (Olivier, 1790)	ZFMK_COL_2009_62	Greven-Bockholt, NW, GER, 2008	GU347104
Bembidion millerianum Heyden, 1883	ZFMK_COL_2009_372	Neheim, NW, GER, 2008	JF895177
Bembidion millerianum Heyden, 1883	ZFMK_COL_2009_373	Neheim, NW, GER, 2008	JF895178
Bembidion millerianum Heyden, 1883	ZFMK_COL_2009_374	Neheim, NW, GER, 2008	JF895180
Bembidion millerianum Heyden, 1883	ZFMK_COL_2009_375	Neheim, NW, GER, 2008	JF895179

Species	ZFMK voucher code	Locality	COI accession number
Bembidion pallidipenne (Illiger, 1802)	ZFMK_COL_2009_67	Fehmarn, SH, GER, 2008	GU347107
Bembidion pallidipenne (Illiger, 1802)	ZFMK_COL_2009_68	Fehmarn, SH, GER, 2008	GU347108
Bembidion pallidipenne (Illiger, 1802)	ZFMK_COL_2009_69	Fehmarn, SH, GER, 2008	GU347109
Bembidion pallidipenne (Illiger, 1802)	ZFMK_COL_2009_70	Fehmarn, SH, GER, 2008	GU347110
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_75	Castrop-Rauxel, NW, GER, 2008	GU347116
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_76	Castrop-Rauxel, NW, GER, 2008	GU347117
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_77	Castrop-Rauxel, NW, GER, 2008	GU347112
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_615	Teutschenthal, ST, GER, 2008	GU347111
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_616	Teutschenthal, ST, GER, 2008	GU347113
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_617	Teutschenthal, ST, GER, 2008	GU347114
Bembidion properans (Stephens, 1828)	ZFMK_COL_2009_618	Teutschenthal, ST, GER, 2008	GU347118
Bembidion punctulatum Drapiez, 1820	ZFMK_COL_2008_39	Bienen, NW, GER, 2006	GU347136
Bembidion punctulatum Drapiez, 1820	ZFMK_COL_2009_382	Dessau, SA, GER, 2008	GU347139
Bembidion punctulatum Drapiez, 1820	ZFMK_COL_2009_383	Dessau, SA, GER, 2008	GU347138
Bembidion punctulatum Drapiez, 1820	ZFMK_COL_2009_384	Neheim, NW, GER, 2008	GU347137
Bembidion pygmaeum (Fabricius, 1792)	ZFMK_COL_2009_385	Halle an der Saale, ST, GER, 2008	JF895173
Bembidion pygmaeum (Fabricius, 1792)	ZFMK_COL_2009_386	Halle an der Saale, ST, GER, 2008	JF895174
Bembidion pygmaeum (Fabricius, 1792)	ZFMK_COL_2009_387	Halle an der Saale, ST, GER, 2008	JF895175
Bembidion pygmaeum (Fabricius, 1792)	ZFMK_COL_2009_388	Halle an der Saale, ST, GER, 2008	JF895176
Bembidion ruficorne Sturm, 1825	ZFMK_COL_2009_391	Feistritz, K, AU, 2008	GU347118
Bembidion ruficorne Sturm, 1825	ZFMK_COL_2009_392	Feistritz, K, AU, 2008	GU347119
Bembidion ruficorne Sturm, 1825	ZFMK_COL_2009_393	Feistritz, K, AU, 2008	GU347120
Bembidion ruficorne Sturm, 1825	ZFMK_COL_2009_394	Feistritz, K, AU, 2008	GU347121
Bembidion semipunctatum (Donovan, 1806)	ZFMK_COL_2008_43	Bienen, NW, GER, 2006	GU347123
Bembidion semipunctatum (Donovan, 1806)	ZFMK_COL_2009_396	Bienen, NW, GER, 2008	GU347122
Bembidion semipunctatum (Donovan, 1806)	ZFMK_COL_2009_397	Bienen, NW, GER, 2008	GU347124
Bembidion semipunctatum (Donovan, 1806)	ZFMK_COL_2009_398	Bienen, NW, GER, 2008	GU347125
Bembidion testaceum (Duftschmid, 1812)	ZFMK_COL_2008_40	Bienen, NW, GER, 2006	JF895166
Bembidion testaceum (Duftschmid, 1812)	ZFMK_COL_2009_401	Feistritz, K, AU, 2008	JF895167
Bembidion testaceum (Duftschmid, 1812)	ZFMK_COL_2009_402	Feistritz, K, AU, 2008	JF895168
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_90	Schmedehausen, NW, GER, 2008	GU347126
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_91	Schmedehausen, NW, GER, 2008	GU347130
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_92	Schmedehausen, NW, GER, 2008	GU347127
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_93	Schmedehausen, NW, GER, 2008	GU347128
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_94	Schmedehausen, NW, GER, 2008	GU347129
Bembidion tetracolum Say, 1823	ZFMK_COL_2009_601	Remagen, RP, GER, 2008	GU347131
Bembidion tibiale (Duftschmid, 1812)	ZFMK_COL_2009_404	Nachrodt-Wiblingwerde, NW,GER, 2008	GU347132
Bembidion tibiale (Duftschmid, 1812)	ZFMK_COL_2009_405	Nachrodt-Wiblingwerde, NW,GER, 2008	GU347133
Bembidion tibiale (Duftschmid, 1812)	ZFMK_COL_2009_406	Zell-Mitterwinkel, K, AU, 2008	GU347134
Bembidion tibiale (Duftschmid, 1812)	ZFMK_COL_2009_407	Zell-Mitterwinkel, K, AU, 2008	GU347135