ARTHROPOD SYSTEMATICS & PHYLOGENY

74 (3): 299–309
13.12.2016

SENCKENBERG

Cryptic diversity in the New World burying beetle fauna: *Nicrophorus hebes* Kirby; new status as a resurrected name (Coleoptera: Silphidae: Nicrophorinae)

Derek S. Sikes *[1], Stephen T. Trumbo[2] & Stewart B. Peck[3]

[1] University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775-6960, USA; Derek S. Sikes* [dssikes@alaska.edu] — [2] University of Connecticut, 99 E. Main St., Waterbury, CT 06702, USA — [3] 993 Normandy Crescent, Ottawa, ON K2C 0L3, Canada — * Corresponding author

Accepted 20.x.2016.
Published online at www.senckenberg.de/arthropod-systematics on 02.xii.2016.

Editor in charge: Christiane Weirauch

Abstract

Burying beetles (Silphidae: *Nicrophorus* Fabricius, 1775) are known for their biparental care and monopolization of small vertebrate carcasses in subterranean crypts. They have been the focus of intense behavioral ecological research since the 1980s and the New World fauna was taxonomically revised in the 1980s. Here, with new molecular, ecological, reproductive incompatability, and morphological data, we report the discovery that *N. vespilloides* in most of North America, except Alaska+Yukon+Northwest Territories, is not conspecific with Old World *N. vespilloides*. DNA barcode data split this species into two BINs, each shows different habitat preferences, most larvae from hybrid crosses fail to reach four days of age, and diagnostic characters were found on the epipleuron and metepisternum that help to separate the species. The oldest available name for this other set of North American populations is *Nicrophorus hebes* Kirby, 1837, which we now treat as valid (**new status**). This study brings the New World total to 22 species for the genus, and given the rarity of *N. hebes*, and its tight association with wetlands, justifies further investigation into its conservation status.

Key words

Burying beetle, *Nicrophorus*, *Nicrophorus vespilloides*, Silphidae, Nicrophorinae, synonymy, Nearctic, DNA barcoding, conservation.

1. Introduction

The genus *Nicrophorus* in the New World was taxonomically revised in the 1980s (Peck & Anderson 1985; Anderson & Peck 1985, 1986) with one new species (*Nicrophorus hispaniola* Sikes & Peck, 2000) added in 2000. Populations of all New World species were sampled for a molecular phylogenetic analysis (Sikes et al. 2008; Sikes & Venables 2013) with the exceptions of *Nicrophorus vespilloides* Herbst, 1783 and *Nicrophorus chilensis* Philippi, 1871. *Nicrophorus vespilloides* is one of the most well studied of the burying beetles, with 635 citations through 2002 (Sikes et al. 2002) and over 1,000 citations found via Google Scholar (10 June 2016). Most of these citations stem from work on Palearctic populations. The Palearctic *N. vespilloides* was also the first silphid to receive whole-genome study (Cunningham et al. 2015; Palmer et al. 2016). The species is Holarctic and relatively high-latitude; most records are north of 40°N. Anderson (1982) documented habitat preferences for silphids in southern Ontario, Canada, and found *N. vespilloides* to be a rarely collected bog/marsh specialist. Purrington & Davidson (2000) documented the southern-most records for this species in North America, from a high elevation acidic *Sphagnum* bog in West Virginia. Beninger & Peck (1992) and Beninger (1994) confirmed these habitat association findings with more extensive study in Ontario, demonstrating that *N. vespilloides* breeds in bog habitats.

Table 1. Specimen and DNA voucher data with BOLD / Genbank #s for COI sequences.

Species	#	Country / Prov	BOLD / Genbank
Nicrophorus tenuipes	01	Japan	EU147484.1
Nicrophorus tenuipes	02	Japan	EU147485.1
Nicrophorus defodiens	03	USA / CT	EU147425.1
Nicrophorus defodiens	04	USA / CO	EU147426.1
Nicrophorus defodiens	05	USA / AK	UAMIC1825-14
Nicrophorus defodiens	06	CAN / NS	BBCEC051-09
Nicrophorus defodiens	07	CAN / AB	SSEIA7772-13
Nicrophorus defodiens	08	CAN / SK	SSPAA5670-13
Nicrophorus defodiens	09	CAN / SK	SSPAB4794-13
Nicrophorus vespilloides	10	RUS	NICRO050-07
Nicrophorus vespilloides	11	Japan	NICRO049-07
Nicrophorus vespilloides	12	CAN / AB	BBCCM046-10
Nicrophorus vespilloides	13	CAN / AB	BBCCM047-10
Nicrophorus vespilloides	14	CAN / NL	BBCEC044-09
Nicrophorus vespilloides	15	Finland	COLFA118-10
Nicrophorus vespilloides	16	Finland	COLFB105-12
Nicrophorus vespilloides	17	Finland	COLFE023-12
Nicrophorus vespilloides	18	Germany	FBCOG508-12
Nicrophorus vespilloides	19	Germany	FBCOH468-12
Nicrophorus vespilloides	20	Germany	FBCOJ052-12
Nicrophorus vespilloides	21	Belgium	FBCOJ394-12
Nicrophorus vespilloides	22	Czech Republic	GBCL4378-09
Nicrophorus vespilloides	23	Germany	GBCOD778-13
Nicrophorus vespilloides	24	Germany	GBCOE852-13
Nicrophorus vespilloides	25	Germany	GBCOG637-13
Nicrophorus vespilloides	26	Germany	GBCOU1133-13
Nicrophorus vespilloides	27	CAN / MB	HMCOC067-07
Nicrophorus vespilloides	28	CAN / AB	TTCFW691-08
Nicrophorus vespilloides	29	USA / AK	UAMIC1835-14
Nicrophorus vespilloides	30	USA / AK	UAMIC2308-14
Nicrophorus vespilloides	31	USA / AK	UAMIC2319-14
Nicrophorus vespilloides	32	USA / AK	UAMIC314-13

This is in contrast to the Palearctic where *N. vespilloides* is relatively easily collected in forests and grasslands (Pukowski 1933; Katakura & Fukuda 1975; Müller & Eggert 1987; Otronen 1988; Scott 1998). These distinct ecological differences between the Palearctic and Nearctic populations were puzzling. It was suggested by Anderson (1985) that the species assemblage of silphids in eastern North America resulted from both ancient (Eocene) and recent (post-Pleistocene glaciation) events. *Nicrophorus vespilloides*, it was presumed, shifted its habitat preferences in the Nearctic to wetland habitats due to competition with its forest-dwelling, and sympatric, sister species *N. defodiens* Mannerheim, 1846 (Anderson 1981; Anderson & Peck 1985; Peck & Anderson 1985; Scott 1998; Sikes & Venables 2013).

Newly available genetic data from the DNA barcode region (Hebert et al. 2003) of the mitochondrial gene COI for *N. vespilloides* from Canada and Alaska divided this species into two groups with the Alaska samples clustering with the Palearctic group. This finding led to investigations, documented herein, into the ecology, morphology, and breeding (in)compatibility of these two groups of beetles to test if they correspond to different biological species (Mayr 2000).

2. Materials and methods

2.1. Phylogenetics

All publicly available *N. vespilloides* CO1 sequences (Canadian, n = 5; Palearctic, n = 14; Alaskan, n = 4) were downloaded from the Barcode of Life Data System or GenBank (Table 1) and combined with downloaded sequences for the sister species, *N. defodiens* (n = 7), and the next closest outgroup taxon, *N. tenuipes* Lewis, 1887 (n = 2) (Sikes & Venables 2013). The data comprised 658 base-pairs and were aligned by eye with reference to amino acids in Mesquite v. 3.03 (Maddison & Maddison 2011) and the best-fitting model was chosen with MrModeltest v2.2 (Nylander 2004). Analyses were run in MrBayes 3.2 under the GTR+I+G model using default priors and settings (2 runs of 4 chains each) for a 1 million step MCMCMC chain with samples taken once every 1000 steps. Stationarity was assessed by ESS values (all parameters had ESS > 354) and Potential Scale Reduction Factors (Gelman & Rubin 1992), which ranged 0.99–1.0. PAUP* 4.0a147 (Swofford 2002) was used to calculate pairwise distances. The Nexus file with data,

MrBayes commands, and resulting tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S20012).

2.2. Ecology

Seven traps baited with rotten chicken wings were set in a dry, upland aspen-birch (*Populus tremuloides – Betula neoalaskana*) hillside in interior Alaska (64.90142°N, 147.5282°W ± 50 m, 280 m elevation, example record from this site with habitat photo: http://arctos.database.museum/guid/UAM:Ento:120751), and run for one night on 14.vi.2014 (7 trap-days of effort). Traps were made from empty 32 oz. plastic yogurt containers nailed to trees at chest height.

In Ontario, three bog/marsh sites similar to, or the same as, those where *N. vespilloides* had been successfully captured by Anderson (1982) or Beninger (1994) were visited. A total of 135 trap-days of effort was expended to collect Canadian *N. vespilloides* using traps baited with three rotten chicken wings each. The three sites and trap dates were: Beetle Acres, Peck cottage, 331 Gibson Road, Newboro, Ontario, edge of *Typha* marsh at forest, 19–22.vi.2014, 44.6277°N 76.3603°W, 123 m elevation, 15 traps nailed to trees at 2 m height (45 trap days); Crosby, Ontario, *Typha* marsh along roadside, 26–29.vi.2014, 44.6550°N 76.2648°W, 123 m elevation, 15 traps set at ground level (45 trap days); and Mer Bleue reserve, Ottawa, Ontario, *Sphagnum* bog, 1–4.vii.2014, 45.3900°N 75.5121°W, 70 m elevation, 15 traps set at ground level (45 trap days).

2.3. Breeding trials

Trials were conducted to determine if there were any pre- or postzygotic reproductive barriers to assess to what degree these populations matched the expectations of the biological species concept of Mayr (2000). Specimens for breeding trials were obtained from the trapping effort described in the preceding section '*Ecology*.' Data from breeding trials are archived at http://dx.doi.org/10.6084/m9.figshare.3569433.

2.3.1. Experiment 1 – Breeding performance. A laboratory colony derived from the Alaska population was started with 7 wild-caught females and 7 wild-caught males. A laboratory colony derived from the Ontario population was started with 1 wild-caught female and 2 wild-caught males. F_1 individuals from both colonies were isolated at adult emergence, kept in small plastic containers (7 cm diameter, 3.5 mm height) at 20°C on a 16L : 8D schedule and fed three times a week on chicken liver scraps. F_1 females were paired with a single F_1 male for 48 h, 2–4 days prior to presentation of a carcass for breeding. Four types of crosses were made to compare the reproductive performance of within population and between population pairings: Alaska female × Alaska male; Ontario female × Ontario male; Alaska female × Ontario male; and Ontario female × Alaska male (N = 7 each). The Ontario × Ontario crosses were between half-siblings because the laboratory population was derived from a single female. To initiate breeding, single females were presented an 18–20 g mouse carcass (Rodent Pro®, Inglefield, IN, U.S.A.) in a covered breeding chamber (35 × 11 × 18 cm) that was half-filled with commercial topsoil and kept in the dark. After 9 days, breeding chambers were checked daily for larval dispersal from the nest. At dispersal, the trial was terminated and the larvae were counted and weighed.

2.3.2. Experiment 2 – Survival of offspring. Experiment 1 indicated that between population pairings were not producing as many larvae or as large a brood mass as within population pairings. To investigate the stage(s) that were affected, second-generation Alaska and Ontario individuals (25–28 days post-emergence) were used. Ontario females were paired with either an Ontario male (N = 16) or an Alaska male (N = 17) and presented a 19–20 g mouse carcass, as above. After 3 days of carcass preparation and oviposition, the male, female and carcass were removed. Chicken liver was placed into the breeding container to attract eclosing first instar larvae. The breeding chamber was checked four times per day and first instars on the liver were removed and placed into small plastic containers (7 cm diameter, 3.5 mm height) with soil and new liver. Survival of larvae was determined through 4 days post-eclosion. At this time, the soil from the original breeding chamber was sifted for eggs that did not hatch.

2.3.3. Experiment 3 – Latency to mate. To compare the willingness of F_1 Ontario females to mate with Ontario and Alaska males, a single female was placed in a small circular arena (9 cm diameter) and allowed 2 minutes to settle. An Ontario (F_1, N = 9) or Alaska (N = 10) male was introduced. The time to copulation was recorded. If a copulation did not occur in 5 minutes, the trial was terminated.

2.3.4. Breeding trial statistical analysis. The reproductive performance of within population and between population pairings (Experiment 1) were assessed using a two-way ANOVA (Least Squares) with the source populations of the female and male as main effects and the specific pairing as the interaction effect (SAS Institute Inc 2007). Zero values for brood mass and number of larvae were included in the analysis as brood failures might indicate between populational incompatibility. The production of eggs, the eclosion rate of first instars and the percentage of first instars that survived 4 days (Experiment 2) were assessed using t tests (zero values included). The frequency of successful copulation (Experiment 3) was assessed using Fisher's Exact test and the latency to copulation by a t test.

Table 2. Museums and their acronyms from which specimens were borrowed for study.

AMNH	American Museum of Natural History, USA	**MZHF**	Zoological Museum, Finland
ANIC	Australian National Insect Collection, Australia	**NHMW**	Naturhistorisches Museum Wien, Austria
BPBM	Bishop Museum, Hawaii, USA	**NSMT**	National Science Museum, Tokyo
BYUC	Monte L. Bean Life Science Museum, Brigham Young University, USA	**PMNH**	Peabody Museum of Natural History, Yale University, USA
CASC	California Academy of Sciences, USA	**RSME**	National Museum of Scotland, UK
CMNC	Canadian Museum of Nature, Canada	**SEMC**	Snow Entomological Museum, USA
CNCI	Canadian National Collection of Insects, Canada	**TAMU**	Insect Collection, Dept. of Entomology, Texas A&M University, USA
DSSC	D. S. Sikes Collection, USA	**TAUI**	Insect Collection, Zoological Museum, Tel Aviv University, Israel
HNHM	Hungarian Natural History Museum, Hungary	**UAM**	University of Alaska Museum Insect Collection, USA
INHS	Illinois Natural History Survey, USA	**UMRM**	Wilbur R. Enns Entomology Museum, University of Missouri, USA
MCZC	Museum of Comparative Zoology, Harvard University, USA	**UNHC**	University of New Hampshire Insect and Arachnid Collections, USA
MNMS	Museo Nacional de Ciencias Naturales, Spain	**ZMUO**	Zoological Museum, University of Oulu, Finland
MSUC	Michigan State University, USA	**ZSMC**	Zoologische Staatssammlung München, Germany
MVMA	Museum of Victoria, Australia		

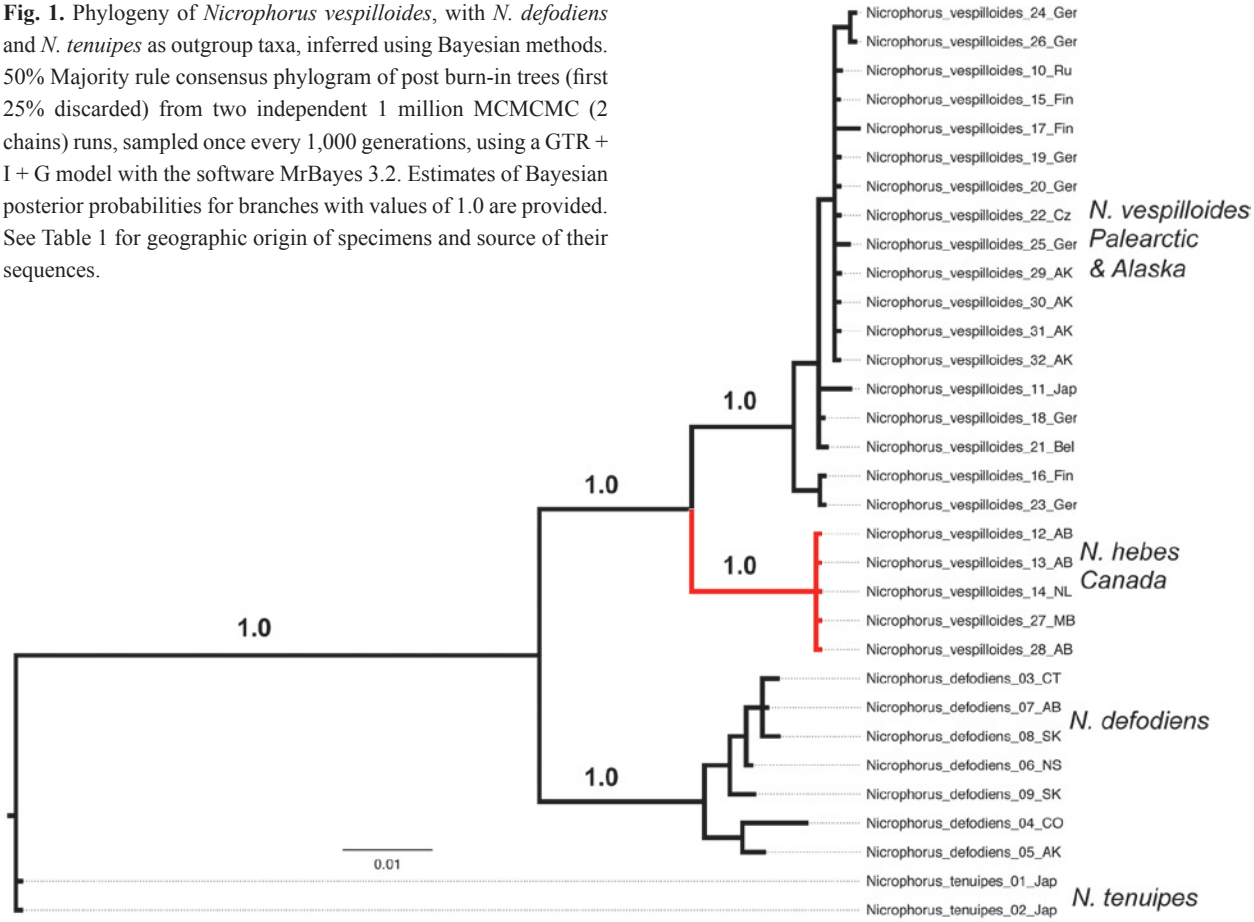

Fig. 1. Phylogeny of *Nicrophorus vespilloides*, with *N. defodiens* and *N. tenuipes* as outgroup taxa, inferred using Bayesian methods. 50% Majority rule consensus phylogram of post burn-in trees (first 25% discarded) from two independent 1 million MCMCMC (2 chains) runs, sampled once every 1,000 generations, using a GTR + I + G model with the software MrBayes 3.2. Estimates of Bayesian posterior probabilities for branches with values of 1.0 are provided. See Table 1 for geographic origin of specimens and source of their sequences.

2.4. Morphology

Nicrophorus vespilloides specimens (n = 1,082) were borrowed from the museums listed in Table 2. Four hundred and fifty of these specimens were from Canada, 178 from Alaska, and 454 from the Palearctic. Examination of all characters commonly used to diagnose *Nicrophorus* species, including various novel characters, resulted in two characters that seemed promising to separate Canadian from Palearctic and Alaskan *N. vespilloides*. All specimens were then sorted into groups for their character states for these two characters prior to examination of locality labels, thus minimizing confirmation bias in scoring of characters. The labels of sorted specimens were then recorded by grouping them into eleven geographic regions (illegible labels and place names that could not be located were ignored): (1) Alaska, (2) Yukon, Northwest Territories, (3) British Columbia, Alberta, Saskatchewan, (4) Nunavut, Manitoba, Ontario, eastern Canada, eastern USA, (5) Spain, (6) UK, (7) Scandinavia, (8) central Europe, (9) southern Europe, Turkey, Israel, (10) central Russia, Mongolia, China, (11) eastern Russia, Japan, Ko-

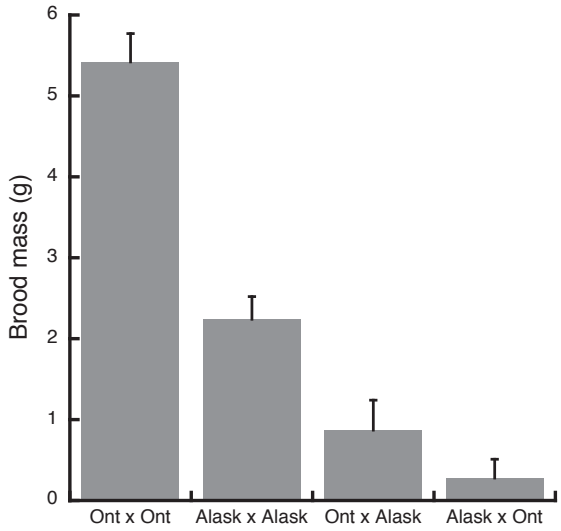

Fig. 2. Brood mass (mean + SE) of four types of pairings of *N. vespilloides* (N = 7).

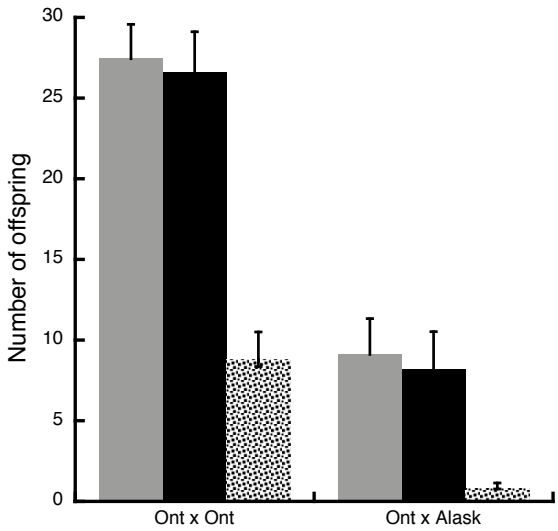

Fig. 3. Number of offspring (mean + SE) at egg (gray bars), first instar (black bars) and larvae at 4 days of age (stippled bars) for crosses between Ontario females and Ontario males versus Ontario females and Alaska males.

rea. Images were captured using a Leica DFC425 camera mounted on a Leica MZ16 stereomicroscope. Updated occurrence data are deposited at figshare.com, https://dx.doi.org/10.6084/m9.figshare.4028358.v1. We downloaded all Nearctic occurrence data in GBIF.org for *N. vespilloides* DOI: http://doi.org/10.15468/dl.jfbe7h. These GBIF-derived data originated from the following collections: EH Strickland Entomological Museum (UASM) University of Alberta, Edmonton, Alberta; Biodiversity Institute of Ontario (BIOUG); C.A. Triplehorn Insect Collection, Ohio State University, Columbus, OH (OSUC); and the Field Museum of Natural History (FMNH). Southern outliers from the distribution in Anderson & Peck (1985) were excluded in the GBIF data for mapping purposes, as they are likely misidentifications of *N. defodiens*. The morphological data used for our analysis are archived at https://dx.doi.org/10.6084/m9.figshare.4007751.v1.

3. Results

3.1. Phylogenetics

Bayesian inference of the COI barcode data found strong support with posterior probabilities of 1.0 for two monophyletic groups within the species *N. vespilloides* (Fig. 1). These groups correspond to samples from Canada *versus* those from Alaska and the Palearctic, which differed (uncorrected 'p' distances) by an average of 3.74% (max = 4.60%, min = 3.19%). These two groups represent different BINs, namely BOLD:AAI3110 and BOLD:AAF3432, in the Barcode of Life Data System (Ratnasingham & Hebert 2007, 2013), which often correspond with species. The Canadian clade showed zero genetic differences among the five sequences despite the wide geographic distances between the samples (Newfoundland, Manitoba, Saskatchewan, Alberta). Within the Palearctic-Alaskan clade, sequences differed by an average of 0.3% (max = 1.2%, min = 0%) across a wide geographic region (Europe – Alaska). *Nicrophorus defodiens* COI sequences differed from *N. vespilloides* sequences by an average of 6.18% (max = 7.94%, min = 5.25%).

3.2. Ecology

One-hundred and thirty five trap-days in Canadian wetlands in Ontario yielded 6 adult *N. vespilloides* (0.0444 adults per trap day). Seven trap-days in Alaskan upland aspen forest yielded 80 adult *N. vespilloides* (11.429 adults per trap day). Although specific to Alaska, this is the first evidence that *N. vespilloides* is dry-forest (non-wetland) associated in the Nearctic.

3.3. Breeding trials

3.3.1. Experiment 1 – Breeding performance. The source population of both the male and female parent significantly affected the total mass of the brood, with Ontario males and females producing heavier broods (Fig. 2). The interaction was also highly significant as between-population pairings produced smaller broods than within-population pairings (Table 3).

3.3.2. Experiment 2 – Survival of offspring. Pairings of Ontario females with Ontario males produced more eggs than pairings of Ontario females with Alaska males ($t31 = 5.95$, $P < 0.0001$; Fig. 3). The eclosion rate

Table 3. Variables explaining reproductive performance (mass of brood, number of larvae, mean mass of larvae) of *N. vespilloides/ N. hebes* (Experiment 1). Significant results in bold.

	Mass of brood		Number of larvae		Mean mass	
Female source population	$F_{1,24} = 18.85$	**P = 0.0002**	$F_{1,24} = 19.79$	**P = 0.0002**	$F_{1,15} = 0.12$	P = 0.74
Male source population	$F_{1,24} = 8.85$	**P = 0.007**	$F_{1,24} = 12.29$	**P = 0.002**	$F_{1,15} = 9.29$	**P = 0.008**
Female x male interaction	$F_{1,24} = 56.32$	**P < 0.0001**	$F_{1,24} = 57.94$	**P < 0.0001**	$F_{1,15} = 0.15$	P = 0.70

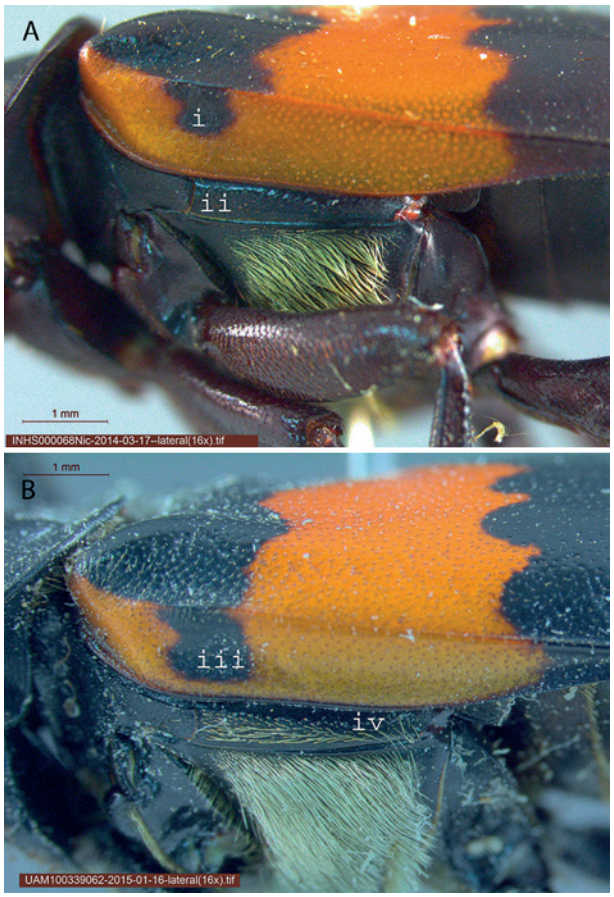

Fig. 4. Left side lateral view of pterothorax of *N. vespilloides* specimens showing **A**: short anterior black band of the epipleuron (i) and bald metepisternum (ii), and **B**: long anterior black band of the epipleuron (iii) and setose metepisternum (iv).

from eggs was high (Ontario × Ontario: 97.0%; Ontario × Alaska: 89.7%) and was not significantly different ($t15.75 = 1.70$, $P = 0.11$). The survival rate of first instars to the third instar without the presence of parents (measured at 4 days post-eclosion) differed by treatment (Ontario × Ontario: 33.1%; Ontario × Alaska: 10.0%) ($t18.56 = 3.41$, $P = 0.003$).

3.3.3. Experiment 3 – Latency to mate. The rate of successful copulation (6 of 10 for Ontario female × Ontario male pairings and 7 of 9 for Ontario × Alaska pairings) was not significantly different (Fisher's Exact test, $P = 0.63$). The latency to copulation (158.83 + 30.26 s for Ontario × Ontario pairings) was not different for Ontario × Alaska pairings (131.57 + 27.50 s) ($t10.63 = 0.67$, $P = 0.52$).

3.4. Morphology

Two characters, each of two states, were found that appeared useful to separate Canadian (except YT, NT) from Alaska+YT+NT+Palearctic *N. vespilloides* adults. The anterior black band of the epipleuron generally is 'short' (crosses less than 75% of the epipleuron, Fig. 4A) or 'long' (crosses 75% or more of the epipleuron, Fig. 4B) and the metepisternum generally is 'bald' (with no, or sparse very short, setae, Fig. 4A) or 'setose' (with few to many long setae, Fig. 4B). Results are presented in Table 4 which indicate that 82.6% of specimens examined show either short anterior bands of the epipleura + bald metepisterna, or long anterior bands of the epipleura + setose metepisterna with the remaining 17.4 % of specimens showing the alternate combinations.

Mapping these character state combinations onto the distribution of *N. vespilloides* (Fig. 5) shows a fairly clear pattern that agrees with the genetic data in supporting two groups, a primarily Canadian (except YT, NT) and an Alaskan+YT+NT+Palearctic group. If these characters were used to predict where a specimen had been collected (i.e. to which group it belongs) these results suggest one would be correct ~ 95% of the time for the majority of specimens (82.6% in our sampling) that show either short + bald or long+setose characters states, and correct approximately ~ 73% of the time for the 17.4% of specimens that show the intermediate character state combinations (Tables 4, 5).

4. Discussion

4.1. Phylogenetics

The phylogenetic and genetic distance analyses, combined with these groups corresponding to two BINs in BOLD, support a hypothesis of two (sister) species. DNA barcodes exist for over 1.8 M specimens and most animal species, based on traditional taxonomy, show greater than 2% divergence from their closest relatives (Ratnasingham & Hebert 2013). We found a greater than 3% divergence between the Canadian and the Alaska + YT + NT + Palearctic groups, which were reciprocally monophyletic (Fig. 1). This is lower than the average among-species genetic distance for the genes COI+COII (~ 7%) in the *Nicrophorus investigator* species group (Sikes et al. 2008) but higher than some among-species distances

Table 4. Number of specimens examined that were categorized into each of four possible state combinations. See text for descriptions of 'short / long' and 'bald / setose.'

States	Count	%
short & bald	306	28.41
long & bald	127	11.79
short & setose	60	5.57
long & setose	584	54.22

Table 5. Percentage of specimens showing the state combination indicated that were collected within the regions listed [e.g. 96.7% of specimens with the states 'short & bald' were collected in Canada (except YT, NT) + NE USA]. See text and Fig. 6 for descriptions of 'short / long' and 'bald / setose.'

States	%	Region
short & bald	96.7	Canada (except YT, NT) + NE USA
long & bald	73.2	Canada (except YT, NT) + NE USA
short & setose	75.0	Alaska + YT + NT + Palearctic
long & setose	98.9	Alaska + YT + NT + Palearctic

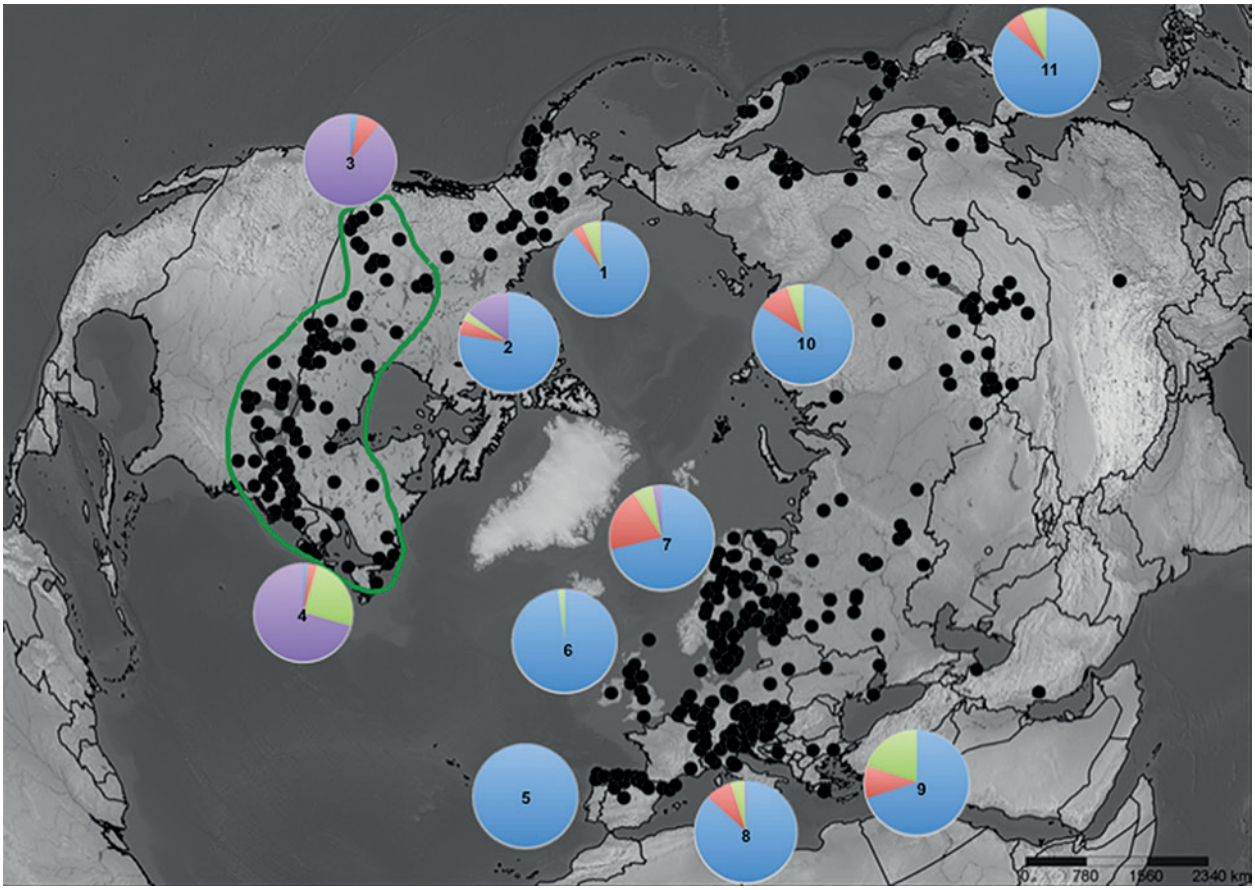

Fig. 5. Pie charts mapped onto distribution of *N. vespilloides* showing percentages of character state combinations (Table 4) within each of eleven geographic regions (1) Alaska, (2) YT, NT, (3) BC, AB, SK, (4) NU, MB, ON, eastern Canada, eastern USA, (5) Spain, (6) UK, (7) Scandinavia, (8) mid-Europe, (9) southern-Europe, Turkey, Israel, (10) mid-Russia, Mongolia, China, (11) eastern Russia, Japan, Korea. Blue = long anterior band of epipleuron + setose metepisternum, purple = short anterior band of epipleuron + bald metepisternum, green = long anterior band of epipleuron + bald metepisternum, red = short anterior band of epipleuron + setose metepisternum. Green line surrounds records identified as conspecific with the Ontario population. Map data available in figshare.com (https://dx.doi.org/10.6084/m9.figshare.4028358.v1). Map prepared using SimpleMappr (Shorthouse 2010).

in the genus (e.g. 0.71%–1.13% between *Nicrophorus nigrita* Mannerheim and *Nicrophorus mexicanus* Matthews; Sikes et al. 2008).

4.2. Ecology

Anderson (1982) captured 32 adult *N. vespilloides* in Ontario, all of which were found in marsh habitats, with none in carrion traps placed in deciduous forests, fields/meadows, or coniferous forests. We therefore targeted wetlands for this species in Ontario. Work on this species in Europe demonstrated its preference for forests (Pukowski 1933; Müller & Eggert 1987; Otronen 1988; Scott 1998), and unpublished data from Alaska indicated this species occurred in forests, so this habitat was targeted in Alaska. We did not attempt to test these prior findings regarding habitat association, which we consider

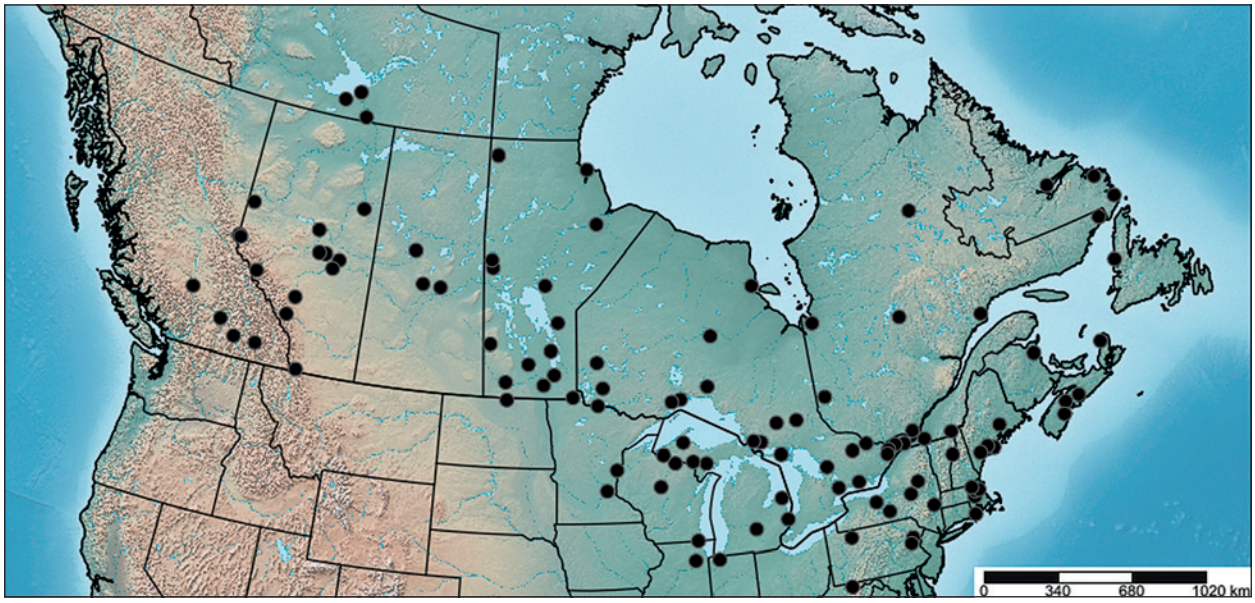

Fig. 6. Locality records for *Nicrophorus vespilloides* now inferred to be *N. hebes* based on literature records (Anderson & Peck 1985; Peck & Kaulbars 1987), examined specimens from museums listed in Table 2, and data downloaded from GBIF.org that passed quality checks (see Methods). Map prepared using SimpleMappr (Shorthouse 2010).

firmly established. Doing so would require trap effort in a variety of habitat types. Rather, we hoped to test prior ecological findings on the commonness, i.e. the trapability, of this species. No prior data have been published demonstrating any population of *N. vespilloides* in North America shows a strong forest (non-wetland) association, which our Alaskan sampling was designed to test.

Anderson (1982) collected a total of 9549 silphid specimens of 12 species over 224 days of collecting with *N. vespilloides* representing a mere 0.3% of the total catch, making it the third rarest species in his study. Anderson's (1982) trap effort in marsh habitat constituted 224 days using 2 pitfall traps, thus representing a total of 448 trap-days. With 32 specimens of *N. vespilloides* collected, this is a rate of 0.0714 *N. vespilloides* per trap-day.

Our results corroborated Anderson's (1982) finding that *N. vespilloides* from Ontario are difficult to capture even when trapping in the preferred habitat. Our results in Ontario found a similar (0.0444 beetles per trap day), but lower rate than Anderson's. We found the Alaskan *N. vespilloides* to be over 250 times more easily trapped (common) than in Ontario, at 11.429 beetles per trap day. This work also documents for the first time a North American population of *N. vespilloides* that is dry forest rather than wetland (bog/marsh) associated.

4.3. Breeding trials

The latency to mate experiment demonstrated that Ontario females will mate with males from either group, suggesting there is no apparent intrinsic pre-zygotic reproductive barrier. Stridulation courtship calls have been shown to be important in *N. mexicanus*; most males who could not stridulate failed to successfully copulate (Huerta et al. 1993). Our results suggest that in *N. vespilloides*, pre-copulatory courtship behavior does not act as a species-isolating mechanism. However, evidence was found for a post-zygotic mating barrier. Three of four counts (brood mass, number of eggs, number of larvae surviving to day 4) were significantly smaller for between population crosses than for within population crosses with only eclosion rate from eggs not differing significantly. This apparent reproductive incompatibility was most pronounced in the number of larvae that reached day four (Fig. 3) which were considerably fewer for between population crosses (mean 0.82 ± 0.32) than for within population crosses (8.8 ± 1.69). The lower production of young from between population crosses suggests that if such matings do rarely happen in regions of sympatry (if such regions exist), that parents would suffer fitness costs. Examination of pre-zygotic barriers in areas of close geographic proximity would be of interest. This is the first attempt in *Nicrophorus* to directly apply the biological species concept (Mayr 2000) and is a satisfying test of the genetic data, including the Barcode of Life Data System's BIN algorithm's (Ratnasingham & Hebert 2007, 2013) relevance to inference of species status.

4.4. Morphology

The morphological characters we found to diagnose these populations do not ensure 100% correct identification of source population and are sometimes hard to judge (e.g. setae can be abraded making a setose metepisternum appear bald, or broken making long setae appear short). However, despite these imperfections, the identification success rate is high enough to be useful, with 82% of specimens examined showing character states

that provide a greater than 95% probability of correctly predicting the source population. Because we were lacking genetic data from populations in Yukon and Northwest Territories we used these morphological characters to assign specimens to source populations and estimate the boundary between populations (Figs. 5, 6). Note that ample keys exist to help separate *N. vespilloides* from *N. defodiens* (e.g. Anderson & Peck 1985) and these new characters are only needed after a positive identification of *N. vespilloides* has been reached for a Nearctic specimen.

4.5. Conclusions

These new findings, combined with prior work demonstrating strong habitat preference differences between the Canadian versus Alaska + YT + NT + Palearctic *Nicrophorus vespilloides*, strongly indicate these groups correspond to different biological species (Mayr 2000). Thus, we herein recognize the oldest available name for the exclusively Nearctic species, *Nicrophorus hebes* Kirby (1837), as valid (**new status**). The type locality of *N. vespilloides* Herbst, 1783 is Berlin, which anchors the name *N. vespilloides* to the Palearctic species.

4.6. Nomenclature & taxonomic history

Kirby (1837) specified the type locality of *N. hebes* as "Nova Scotia" and provided a common name for the species: "Unsensed Necrophorus." He diagnosed the species on the basis of it lacking a 'rhinarium,' (which he also called a nose and a nostril piece) now known as the clypeal membrane, which is puzzling because the holotype, a large male, has a large clypeal membrane, albeit a black one (*N. vespilloides* and *N. hebes* are two of five species in the genus with black clypeal membranes, the rest have easily seen orange, yellow, or brown membranes. Perhaps Kirby thought it absent because it was difficult to see). Kirby likely thought the clypeal membrane functioned as a sensory organ and therefore applied the epithet '*hebes*' which means, in relation to the senses, "dim, faint, dull; tasteless, without smell" in Latin. The name *Nicrophorus hebes* was considered a valid species in at least six publications since the original description until Crotch (1873) synonymized it as a variety of *N. vespilloides* where it remained until it was demoted to a rankless synonym of *N. vespilloides* by Horn (1880). The name *N. hebes* was listed as a synonym of *N. vespilloides* in at least 11 other publications since Horn (Sikes et al. 2002), including the taxonomic revision of the Nearctic Silphidae by Anderson & Peck (1985), although Portevin (1926) and Hatch (1928) listed it as a synonym of *N. defodiens*. However, as is unfortunately typical of much taxonomic work, it is not clear from these publications if the authors were simply repeating the conclusions of prior authors without attribution, or if they had studied the *N. hebes* type specimen and came to the same conclusion as prior authors (most likely the former). The first author has examined the *N. hebes* holotype in the Natural History Museum in London and asked Maxwell Barclay, curator of Coleoptera there, to double-check these characters. The holotype is missing its left elytron but the right elytron has a black band that covers less than 75% of the epipleuron (= "short") and the metepisternum appears bald (pers. comm. 1 July 2016 M. Barclay). Kirby's (1837) understanding of sexual dimorphism and variation within and among species in the genus *Nicrophorus* was clearly superficial – his naming of *N. hebes* was essentially accidental since his diagnosis cannot be used to separate *N. hebes* from *N. vespilloides*. Perhaps he was partially motivated to name this species by the reasonable hypothesis that, although similar to *N. vespilloides* in the Palearctic, the geographic distance and ocean barrier to dispersal would reduce the likelihood of conspecifity. However, and not without a touch of irony, Kirby accidentally provided a name, the 'Unsensed *Nicrophorus*,' which is quite appropriate for a cryptic species that has remained undetected for over a century.

Kirby (1837) also described *Nicrophorus pygmaeus* Kirby, from a single specimen taken in the rather vaguely specified "N. Amer. Rocky Mountains." The first author studied the holotype of *N. pygmaeus* in the Natural History Museum in London and confirmed the name as a synonym of *N. vespilloides* (Sikes et al. 2002), it having first been synonymized by LeConte (1870) under *N. defodiens*, and later moved under *N. vespilloides* by Crotch (1873). The holotype of *N. pygmaeus* bears the character states of *N. vespilloides* (long black band of epipleuron, setose metepisternum [pers. comm. M. Barclay]), not *N. hebes*, and, given the type locality, could have been collected from the western border of Northwest Territories (Lindroth 1953), where *N. vespilloides* occurs. There remains a chance that the type specimens of *N. pygmaeus* and *N. hebes* are conspecific; if this turns out to be the case, following article 24.2.1 of the International Code of Zoological Nomenclature (4th ed.) we choose as first revisers the name *N. hebes* for this species.

4.7. How confident are we that these species are not sympatric?

Given the rarity and difficulty of collecting *N. hebes*, if *N. vespilloides*, which is much easier to collect, was sympatric with *N. hebes*, the sampling effort which resulted in the small series of disparately collected specimens that were DNA barcoded would almost certainly have detected *N. vespilloides* from the range of *N. hebes*. This did not happen. Additionally, Anderson (1982), Beninger (1994) and our efforts would have found *N. vespilloides* in both forest and wetlands in Ontario, which did not happen. To date, there is no evidence that *N. vespilloides* occurs east of about 120° longitude or south of about 61° latitude in the Nearctic (Fig. 5). However, it is unclear if, or to what degree, these species' ranges overlap in western Canada (most likely in northwestern Alberta).

4.8. Evolutionary and biogeographic considerations

These results suggest a model of multiple dispersal and speciation events between the New (NW) and Old Worlds (OW). The analysis of Sikes & Venables (2013) found evidence that the most recent common ancestor (MRCA) of *N. vespilloides* and *N. defodiens* was NW and that the OW population of *N. vespilloides* resulted from dispersal to the OW from the NW of the forest-associated MRCA. The alternative, that the MRCA was OW, had less statistical support, but is actually more parsimonious because the favored scenario also predicts *N. tenuipes* to result from NW to OW dispersal – requiring two dispersal and speciation events whereas an OW MRCA would require only one dispersal and speciation event. In any case, these two species, *N. vespilloides* and *N. defodiens*, were estimated to have originated from a speciation event ~ 10–30 Mya.

Our current findings agree with the following biogeographic scenario to explain the presence of these three closely related species in the NW – that, once isolated, the forest dwelling MRCA became the species we call *N. vespilloides* in the OW with this niche filled by *N. defodiens* in the NW. Subsequently, *N. vespilloides* dispersed again into the NW from the OW, but competition with *N. defodiens* selected for a variety of *N. vespilloides* that could survive in marginal habitat (bogs and marshes). This population was genetically isolated from OW *N. vespilloides* for a long enough time period, which included Pleistocene glaciations that likely forced populations southward, to become a distinct sister species, *N. hebes*. The genetic data support this in indicating *N. hebes* may have experienced a bottleneck that greatly reduced its genetic diversity. Most recently, presumably when the Bering land bridge was present during the Pleistocene, forest-dwelling *N. vespilloides* from eastern Asia dispersed into Alaska and northwestern Canada. This population appears to be slightly sympatric with *N. defodiens* in south-central Alaska which would be an ideal location to study their potential interaction.

4.9. Remaining questions

There are many interesting questions for future study. How consistent is the bog-habitat association throughout the full range of *N. hebes*? Is *N. hebes* consistently rare throughout its range? Do these species occur in sympatry in northwestern Canada? Do they maintain tight habitat associations there? Is there some gene flow between these species? If both species co-occur in northwestern Canada do they show the same reproductive failures when cross bred? Are there more reliable morphological characters (of adults and larvae) to separate these sister species?

5. Acknowledgments

Funding for the DNA barcoding project, which generated the Alaskan genetic data, was provided by the United States Fish and Wildlife's Alaska Region NWRS Inventory and Monitoring Initiative. We thank Ronald Madge for reviewing an earlier draft of the manuscript and helping with the etymology of the epithet *hebes*. We thank Max Barclay, Curator and Collection Manager of Coleoptera and Hemiptera at the Natural History Museum in London, for confirming character states of the Kirby type specimens.

6. References

Anderson R.S. 1981. The biology and distribution of the Silphidae and Agyrtidae of Canada and Alaska. – M.Sc. thesis, Carleton University, Ottawa, Ont., Canada.

Anderson R.S. 1982. Resource partitioning in the carrion beetle (Coleoptera: Silphidae) fauna of southern Ontario: ecological and evolutionary considerations. – Canadian Journal of Zoology **60**: 1314–1325.

Anderson R.S., Peck S.B. 1985. The Carrion Beetles of Canada and Alaska (Coleoptera: Silphidae and Agyrtidae). The Insects and Arachnids of Canada, Part 13. – Publication 1778, Research Branch Agriculture Canada, Ottawa. 121 pp.

Anderson R.S., Peck S.B. 1986. Geographic patterns of colour variation in North American *Nicrophorus* burying beetles (Coleoptera; Silphidae). – Journal of Natural History **20**: 283–297.

Beninger C.W., Peck S.B. 1992. Temporal and spatial patterns of resource use among *Nicrophorus* carrion beetles (Coleoptera: Silphidae) in a Sphagnum Ontario and adjacent forest near Ottawa, Canada. – Canadian Entomologist **124**: 79–86. doi: http://dx.doi.org/10.4039/Ent12479-1

Beninger C.W. 1994. Phenology, reproductive biology and habitat associations of *Nicrophorus* Fab. (Coleoptera: Silphidae) of the Mer Bleue Ontario area (Ottawa, Canada). – Memoirs of the Entomological Society of Canada **126**: 135–143. doi:10.4039/entm126169135-1

Crotch G.R. 1873. Check List of the Coleoptera of America, North of Mexico. – Naturalists' Agency, Salem, Mass. 136 pp.

Cunningham C.B, Ji L., Wiberg R.A.W., Shelton J.M., McKinney E.C., Parker D.J., Meagher R.B., Benowitz K.M., Roy-Zokan E.M., Ritchie M.G., Brown S.J., Schmitz R.J., Moore A.J. 2015. The genome and methylome of a beetle with complex social behavior, *Nicrophorus vespilloides* (Coleoptera: Silphidae). – Genome Biology and Evolution **7**: 3383–3396. doi:10.1093/gbe/evv194

Gelman A., Rubin D. 1992. Inference from iterative simulation using multiple sequences. – Statistical Science **7**: 457–472.

Hebert P.D., Cywinska A., Ball S.L. 2003. Biological identifications through DNA barcodes. – Proceedings of the Royal Society of London B: Biological Sciences **270**(1512): 313–321.

Horn G.H. 1880. Synopsis of the Silphidae of the United States with reference to the genera of other countries. – Transactions of the American Entomological Society **8**: 219–322.

Huerta C., Halffter G., Fresneau D. 1993. Inhibition of stridulation in *Nicrophorus* (Coleoptera: Silphidae): Consequences for reproduction. – Elytron **6**: 151–157.

Katakura H., Fukuda H. 1975. Faunal makeup of ground and carrion beetles in Komiotoineppu, Hokkaido University Naka-

gawa Experimental Forest, northern Japan, with some notes on related problems. – Research Bulletins of the College Experiment Forests Hokkaido University **32**: 75–92.

Kirby W. 1837. The Insects. In: Richardson J. (ed.), Fauna Boreali-Americana, or the Zoology of the Northern Parts of British America. Part 4. – J. Fletcher, Norwich. 325 pp., 8 pls.

LeConte J.L. 1870. Synonymical notes on North American Coleoptera. – Annals and Magazine of Natural History **4**: 394–404.

Lindroth C.H. 1953. Kirby's types of North American Carabidae (Coleoptera). – Proceedings of the Royal Entomological Society of London, Series B: Taxon **22**: 167–177.

Maddison W.P., Maddison D.R. 2011. Mesquite: a Modular System for Evolutionary Analysis. Version 3.03. – http://mesquiteproject.org.

Mayr E. 2000. The Biological Species Concept. Pp. 17–29 in: Wheeler Q., Meier R. (eds), Species Concepts and Phylogenetic Theory: a Debate. – Columbia University Press, New York.

Müller J.K., Eggert A.-K. 1987. Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). – Ethology **76**: 297–304.

Nylander J.A.A. 2004. MrModeltest v2. – Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Otronen M. 1988. The effects of body size on the outcome of fights in burying beetles (*Nicrophorus*). – Annales Zoologici Fennici **25**: 191–201.

Palmer W.J., Duarte A., Schrader M., Day J.P., Kilner R., Jiggins F.M. 2016. A gene associated with social immunity in the burying beetle *Nicrophorus vespilloides*. – Proceedings of the Royal Society B **283**: 20152733. http://dx.doi.org/10.1098/rspb.2015.2733

Peck S.B., Anderson R.S. 1985. Taxonomy, phylogeny and biogeography of the carrion beetles of Latin America (Coleoptera: Silphidae). – Quaestiones Entomologicae **21**: 247–317.

Peck S.B., Kaulbars M.M. 1987. A synopsis of the distribution and bionomics of the carrion beetles (Coleoptera: Silphidae) of the conterminous United States. – Proceedings of the Entomological Society of Ontario **118**: 47–81.

Portevin G. 1926. Les Grands Nécrophages du Globe. Silphini – Necrodini – Necrophorini. Encyclopédie Entomologique (A), Vol. 6. – Lechevalier, Paris. 270 pp.

Pukowski E. 1933. Ökologische Untersuchungen an *Necrophorus* F. – Zeitschrift für Morphologie und Ökologie der Tiere **27**: 518–586.

Purrington F.F., Davidson R.L. 2000. New southerly distribution records for the boreal carrion beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). – Entomological News **111**: 355–358.

Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). – Molecular Ecology Notes **7**: 355–364. doi: 10.1111/j.1471-8286.2006.01678.x

Ratnasingham S., Hebert P.D.N. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. – PLoS ONE **8**: e66213. doi:10.1371/journal.pone.0066213

SAS Institute Inc. 2007. JMP statistics and graphics guide v. 7. – Cary, NC: SAS Institute, Inc.

Scott M.P. 1998. The ecology and behavior of burying beetles. – Annual Review of Entomology **43**: 595–618.

Shorthouse D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. [Retrieved from http://www.simplemappr.net. Accessed 17 March 2014.]

Sikes D.S., Madge R.B., Newton A.F. 2002. A catalog of the Nicrophorinae (Coleoptera: Silphidae) of the world. – Zootaxa **65**: 1–304.

Sikes D.S., Vamosi S.M., Trumbo S.T., Ricketts M., Venables C. 2008. Molecular systematics and biogeography of *Nicrophorus* in part – the *investigator* species group (Coleoptera: Silphidae) using mixture model MCMC. – Molecular Phylogenetics and Evolution **48**: 646–666. http://dx.doi.org/10.1016/j.ympev.2008.04.034

Sikes D.S., Venables C. 2013. Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). – Molecular Phylogenetics and Evolution **69**: 552–565. http://dx.doi.org/10.1016/j.ympev.2013.07.022

Swofford D.L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. – Sinauer Associates, Sunderland, Massachusetts.