

# Cooled Propylene Glycol as a Pragmatic Choice for Preservation of DNA From Remote Field-Collected Diptera for Next-Generation Sequence Analysis

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## Abstract

Next-generation sequencing (NGS)-based methods can now be applied to large population-scale studies, but this demands very high-quality DNA. For specimens collected from remote field locations, DNA degradation can be a problem, requiring logistically challenging preservation techniques. Simpler preservation techniques are therefore required. Prior to collection of exotic fruit fly (Tephritidae) species, a number of readily available preservatives with storage at either 4°C or room temperature were trialed here to determine the DNA quality for three locally available Diptera species, *Fannia canicularis* (L.), *Musca domestica* L., and *Lucilia sericata* Meigen. Considerable variation was observed between the different preservatives, species, and temperatures, but several preservatives at 4°C were favored. Chilled propylene glycol was subsequently used for the storage and carriage of Australian field-collected *Bactrocera* fruit fly specimens to New Zealand. When processed up to 20 d later, DNA fragments of ~10–20 kb were obtained for successful genotyping by sequencing analysis. This protocol is therefore recommended as a logistically simple and safe approach for distant collection of dipteran samples for NGS population genomic studies.

**Key words:** genotyping by sequencing, GBS, Tephritidae, next-generation sequencing, DNA preservation

Advances in next-generation sequencing (NGS) technology have enabled the large-scale capture of genome-wide inter- and intraspecific DNA variation. This has facilitated the emergence of population genomic studies that were not previously possible using standard Sanger sequencing of single- or multiloci (Cosart et al. 2011, Straub et al. 2012, McCormack et al. 2013). As a consequence, more sophisticated questions can be asked in ecological genetics (Hecht et al. 2013) and phylogenetics (Emerson et al. 2010, Nadeau et al. 2013, Cruaud et al. 2014), including for nonmodel organisms with large genomes (Hohenlohe et al. 2010, Nadeau et al. 2013). Despite these technical advances, the requirement for very high quality and quantity of DNA for NGS (e.g., Hogan et al. 2008, Rizzo and Buck 2012) can severely limit its application to wild populations, especially where broad geographic coverage is necessary to capture genetic variation. Keeping specimens alive from remote field collection sites until they reach the laboratory is rarely practical, especially for exotic species, but to maintain them dead can severely impact on the quality of the DNA. Optimal preservation of field-collected forensic samples for DNA analysis has been considered using standard preservatives such as alcohols, RNAlater, and silica (Michaud and Foran 2011). However, while the DNA quality they achieved was suitable for PCR, it would not have been acceptable for NGS. Hohenlohe et al. (2010) placed freshly collected sticklebacks onto

dry ice, but this is logistically very complex with time and air travel constraints; the latter also an issue for many chemical preservatives judged toxic or flammable (Williams 2007). For NGS studies, those analyzing insects have either used laboratory colonies (e.g., Baxter et al. 2011), or simply do not mention how specimens were collected and stored (e.g., Emerson et al. 2010, Lozier 2014). Essentially, there is a general lack of literature on how to collect insect specimens from wild populations for NGS analysis.

As part of a wider project studying species divergence and population-level variation within the *Bactrocera tryoni* (Froggatt) species complex (Diptera: Tephritidae), fresh samples from populations across very large areas of Australia were to be acquired. These were then to be analyzed by genotyping by sequencing (GBS; Elshire et al. 2011) at a genetics laboratory in New Zealand. Based on the collection, handling, and storage method previously used for single gene and microsatellite studies of other *Bactrocera* species (Schutze et al. 2012, Krosch et al. 2013, Boykin et al. 2014), initial samples were collected directly into propylene glycol. These were maintained at ambient temperature (~20–30°C) for 7–60 d until they could be returned to the laboratory where they were immediately transferred into ethanol. However, while the DNA of these flies was suitable for amplicon sequencing of the COX1 gene, it was of insufficient quality for GBS, which requires average fragment lengths of ~20 kb

(<http://www.biotech.cornell.edu/brc/genomic-diversity-facility/gbs-sample-submission> 13 August 2015). To overcome this, subsequent collaboration with an Australian-based laboratory enabled flies to be maintained live or freshly killed (<24 h) prior to freezing at  $-20^{\circ}\text{C}$  and DNA extractions completed there. This was a successful strategy for GBS, but similar arrangements were not possible for other, more remote population locations. Alternative in-field DNA extraction methods such as FTA cards (Whatman; Smith and Burgoyne 2004) or *Xpedition* (Zymo Research) were considered, being suitable for small samples under nonrefrigerated field conditions. However, these destructive methods would need to be carried out in-field by experts capable of prior species identification to distinguish the correct species from the several that are attracted to the generic lure (cue-lure)-baited traps; travel by an expert to all locations was impractical.

Given this, an investigation was undertaken here to find an appropriate protocol for collection of field material by nontaxonomic experts, and return to New Zealand with minimal impact on DNA quality. The focus was on chemicals which would be common in any genetics laboratory and considered safe for air transportation. As dachne fruit flies are not present in New Zealand, and considered a high biosecurity risk, this testing required use of locally available species, of which *Fannia canicularis* (L.) (Diptera: Fanniidae) the lesser house fly, *Musca domestica* L. (Diptera: Muscidae) the house fly, and *Lucilia sericata* Meigen (Diptera: Calliphoridae) the blow fly, were selected. The preferred protocol was then validated with fresh collections of *B. tryoni* complex specimens from Australia.

## Materials and Methods

### Pilot Storage Medium Experiment Using *F. canicularis*

Specimens of *F. canicularis* were collected in Lincoln (NZ) and placed individually, live, into 1.7-ml microcentrifuge tubes each containing 180  $\mu\text{l}$  of either: 1) 99.5% propylene glycol (Sigma-Aldrich); 2) Qiagen Buffer AL (containing guanidine hydrochloride); 3) Qiagen Buffer ATL (lysis buffer containing SDS, suggested for tissue storage); 4) *RNAlater* (Ambion); 5) phosphate buffered saline (PBS) pH 7.4 (Sigma); 6) Qiagen Buffer AE (TE); 7) Qiagen RNA-free  $\text{H}_2\text{O}$  (negative control); 8) RNA lysis buffer (Promega); and 9) 97–100% ethanol. Qiagen buffers were from the DNeasy Blood and Tissue DNA extraction kit. Five specimens were prepared for each treatment. These were then stored at  $4^{\circ}\text{C}$  as a temperature attainable with the domestic refrigerators or ice in insulated containers that are feasible options for remote field collections. In addition, five specimens were similarly stored frozen dry at  $-20^{\circ}\text{C}$  as a positive control. Each specimen was then processed at either 1, 8, 13, 14, or 15 d after storage. Immediately before DNA extraction they were placed onto tissue paper for 5 min to remove the storage medium. DNA was extracted from whole flies using the Qiagen DNeasy Blood & Tissue Kit, which is recommended for maintaining large fragments of predominately  $\sim 30$  kb (Qiagen 2006). The supplementary protocol for insects was followed, but only 70  $\mu\text{l}$  of elution buffer AE was used instead of the recommended 200  $\mu\text{l}$  to improve the DNA concentration. As required by the sequencing laboratory, initial quality and quantity of the DNA was determined using 2  $\mu\text{l}$  of each sample electrophoresed on a 1% agarose gel, along with 5  $\mu\text{l}$  of the  $\lambda$  DNA/Hind III Fragments ladder (Invitrogen), and visualized with Red Safe Nucleic Acid staining solution (iNtRON Biotechnologies).

### Comprehensive Storage Medium Experiment Using *M. domestica* and *L. sericata*

Further testing, using only the seasonally independent, commercially available *M. domestica* and *L. sericata*, followed the same protocol as above; this with the exception that Qiagen Buffer AE and Qiagen RNA-free  $\text{H}_2\text{O}$  were excluded, and dried and fresh flies of each species were included as additional positive controls. Dried samples were killed by freezing at  $-20^{\circ}\text{C}$  for a few hours, then stored at each of two temperatures (room temperature  $\sim 20^{\circ}\text{C}$  and  $+4^{\circ}\text{C}$ ) and three storage times (1, 8, and 16 d). Three specimens were prepared for each combination of 8 treatments  $\times$  2 species  $\times$  3 storage time  $\times$  2 storage temperatures, totaling 36 samples per treatment and 96 per storage time. Quality of the DNA was observed by electrophoresis as above. Total DNA concentration was analyzed spectrofluorimetrically using a Quant-iT PicoGreen dsDNA Assay kit (Molecular Probes) on a FluoSTAR Omega fluorimeter (BMG labtech), in a 200  $\mu\text{l}$  assay volume using flat-bottomed black Nunclon<sup>TM</sup> Delta Surface 96-well plates (Thermo Scientific). A  $1 \mu\text{g/ml}$ – $0.1 \text{ ng/ml}$  standard curve was prepared using  $\lambda$  DNA according to manufacturer's recommendations. Data were considered accurate when raw data readings between replicates differed by  $<5\%$ , and  $R^2$  of a standard curve was  $0.99$ – $1$ . For further analysis, blank-corrected data were used (generated automatically by MARS data analysis software 1.20 R3). Genstat v17.1 (Payne 2009) was used to conduct an analysis of variance (ANOVA) test on the resulting data with the two temperature treatments treated as separate mini trials.

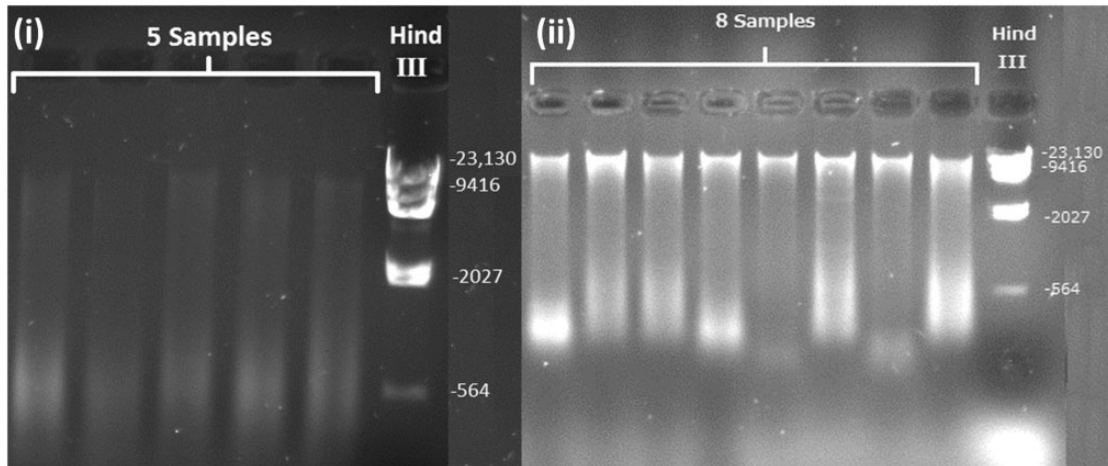
### Validation of the Preferred Technique, Cooled Propylene Glycol

Specimens of *B. tryoni* complex flies (*B. tryoni* and *Bactrocera neohumeralis* (Hardy)) were collected from March to April 2014 in north Queensland and central New South Wales. Live flies were collected in cue-lure-baited modified Steiner traps (Drew et al. 1978) and returned in the traps to local accommodation, where they were cooled in a refrigerator before preliminary sorting and identification. Based on the results of the previous laboratory experiments, these flies were stored in individual microcentrifuge tubes containing propylene glycol and maintained as cold as possible in either the ice shelf of a bar fridge or the freezer component of a fridge freezer. When moving between field locations, and for air travel back to New Zealand, the tubes were contained in a small standard vacuum flask containing ice. All samples were taken back to New Zealand within 20 d of collection and then placed in a  $-80^{\circ}\text{C}$  freezer. DNA extractions occurred within one month of returning to New Zealand using the protocols described in the pilot experiment. DNA extractions were frozen at  $-20^{\circ}\text{C}$  on the day of extraction. Evidence of the quality and quantity of DNA was submitted to the Cornell University Institute for Genomic Diversity GBS submission page (<http://sorghumdiversity.maize.cornell.edu>) as electrophoresis gel images to confirm presence of high molecular weight DNA ( $\sim 20$  kb) at  $50$ – $100 \text{ ng}/\mu\text{l}$  (concentrations  $<10 \text{ ng}/\mu\text{l}$  are not recommended). Accurate sizing and quantitation was subsequently carried out there using an Agilent 2100 Bioanalyzer prior to GBS analysis.

## Results

### Pilot Storage Medium Experiments Using *F. canicularis*

Genomic DNA of *F. canicularis* specimens stored at  $4^{\circ}\text{C}$  in RNA-free  $\text{H}_2\text{O}$ , Buffer AE, or PBS produced weak or no high molecular weight banding when run on agarose gels, implying that it had largely degraded. From the remaining treatments, the strongest



**Fig. 1.** Example taken of DNA extracts from (i) five field-collected *B. tryoni* held in propylene glycol and stored at room temperature until DNA extraction and (ii) eight field-collected *Bactrocera tryoni* held in propylene glycol and kept as cool as possible during field collection and return to the laboratory. Hind III refers to the TrackIt DNA/Hind III Fragment DNA ladder (Invitrogen) run with the DNA extractions with the size of four fragments labeled.

bands were from those specimens stored in Buffer AL and propylene glycol, and for which the DNA did not appear to degrade over time (Supp. Fig. 1 [online only]).

### Comprehensive Storage Medium Experiment Using *M. domestica* and *L. sericata*

Spectrofluorometric measurements of total DNA concentrations retrieved from flies in the different storage media across three time periods are illustrated for the two species in Supp. Fig. 2 (online only). This reveals considerable variation within and between all treatments, and which often overlapped within a species across the time and storage treatments. Various unrelated reasons may have contributed to this level of variation, including operational factors, such as unavoidable inconsistency in elution of DNA from the extraction columns using the reduced volume of elution buffer. The ANOVA test revealed significant differences between some preservatives when the species and time treatments were combined for each preservative and temperature treatment; ethanol had the highest average DNA concentration for both of these (Supp. Fig. 3 [online only]). DNA concentrations in ethanol were not significantly different from the next best preservative RNALater at room temperature, but were significantly different to the next best preservative propylene glycol at 4°C. DNA concentration in ethanol was also significantly higher than propylene glycol at room temperature. Importantly, all treatments produced some appropriately high molecular weight DNA (data not shown) as exemplified in Supp. Fig. 1 (online only), even though some degradation of DNA was apparent for the majority of samples.

### Validation of Preferred Technique, Cooled Propylene Glycol

Propylene glycol was chosen as the storage medium for a field validation trial. Based on the laboratory test above, this did not preserve total DNA any better than other treatments, or have any obviously greater amounts of high molecular weight DNA. However, compared with the other media, it is either cheaper, more easily obtained, or can be transported through customs and on airplanes without concern (Nagy 2010). Of the DNA extracted from 148 specimens, 99 (69%) produced a bright band between the 9,416 bp and 23,130 bp fragments of the

DNA/Hind III molecular weight ladder (Invitrogen; Fig. 1). Subsequent quality control data using a Bioanalyzer confirmed the libraries to be composed of sufficient quantities of fragments >10 kb. All samples submitted for GBS analysis have been successfully sequenced (data not shown) and aligned to a *B. tryoni* reference genome to confirm successful extraction of the target DNA.

### Discussion

Several preservatives, including within commonly encountered buffers found in extraction kits, have been shown here to be suitable for the storage of fly specimens for subsequent high molecular weight DNA extraction. These are compared with the “gold standards” of ethanol and freezing, both of which are highly impractical for field collection work. Preservation of tissues to minimize DNA degradation commonly utilizes the inactivation of nucleases. Accordingly, the chaotropic salts guanidine hydrochloride in buffer AL (DNeasy Blood & Tissue Handbook, Qiagen) and guanidinium thiocyanate in RNALater and the RNA lysis buffer are used to denature proteins, including nucleases (Salvi et al. 2005). Hence, the former has been found to be a good temporary transport medium for the preservation of sponge (Porifera) DNA (Salgado et al. 2007). However, it has been noted elsewhere that RNALater for field-collected samples is expensive, not easily accessible, and can interfere with some DNA extraction methods (Michaud and Foran 2011). The proprietary buffer ATL containing SDS, which is also a protein denaturant (Bhuyan 2010), is likewise noted as a good tissue preservative for high-quality DNA extraction (DNeasy Blood & Tissue Handbook, Qiagen). Surprisingly, while PBS has no anticipated preservative qualities, it has been shown to be more effective than propylene glycol for hard-bodied beetles (Stevens et al. 2011).

Propylene glycol was chosen for validation based on the practical attributes of accessibility and price as well as the nontoxicity and nonflammability requirements for air transport. Subsequent ease of use of the method has been evidenced by receipt of fruit fly samples from remote areas in far northwestern Australia. Packed in ice-filled, locally available vacuum flasks, flies in propylene glycol were simply posted to New Zealand with no apparent DNA degradation, and successful analysis by GBS. The alternative use of dried flies, as

suggested by the laboratory results here, was not considered given the difficulty keeping specimens dry under field conditions.

The hygroscopic nature of propylene glycol, as with ethanol, dehydrates tissues to remove the water necessary for enzyme activity (Prestrelski et al. 1993). Hence, it is well known for its ability to preserve PCR-amplifiable insect tissue (e.g., Stevens et al. 2011, Schutze et al. 2012, Ferro and Park 2013, Moreau et al. 2013). However, a previous assessment of propylene glycol, by Vink et al. (2005), found that storing field-collected spiders and scorpions in propylene glycol and RNAlater resulted in significantly higher quality DNA compared with various ethanol concentrations. Their data also suggested that preservation in propylene glycol at 19–24°C, 2–4°C, or –20°C would provide the DNA quality considered suitable for GBS. Although interestingly similar preservation at the more extreme temperatures of 40°C or –80°C would not. Certainly the importance of temperature for field collection was also implied in the current study, given the initial collection of fruit flies using ambient temperature propylene glycol were not suitable for NGS, but specimens of the same species collected in the same way using cooled propylene glycol were.

In summary, we have demonstrated that DNA suitable for NGS analyses can be produced for medium-sized Diptera from remote field collection sites if a very simple technique for preservation is followed. Therefore, the assistance of nonexperts in the field, using nontoxic and readily available propylene glycol, locally purchasable vacuum flasks, and ice from a hotel bar-fridge makes the ability to maintain quality DNA more feasible. Consequently, this approach could significantly enhance the potential for NGS-related studies of natural populations. However, the variability in DNA yields revealed here between preservatives, temperature, and species, suggests that factors beyond those could impact on the success rate. Therefore, tests like this are recommended before embarking on expensive collection expeditions and analyses.

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## Supplementary data

Supplementary data are available at Journal of Economic Entomology online.

## References Cited

- Baxter, S. W., J. W. Davey, J. S. Johnston, A. M. Shelton, D. G. Heckel, C. D. Jiggins, and M. L. Blaxter. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. *PLoS ONE* 6: e19315.
- Bhuyan, A. K. 2010. On the mechanism of SDS-induced protein denaturation. *Biopolymers* 93: 186–199.
- Boykin, L. M., M. K. Schutze, M. N. Krosch, A. Chomič, T. A. Chapman, A. Englezou, K. F. Armstrong, A. R. Clarke, D. Hailstones, and S. L. Cameron. 2014. Multi-gene phylogenetic analysis of south-east Asian pest members of the *Bactrocera dorsalis* species complex (Diptera: Tephritidae) does not support current taxonomy. *J. Appl. Entomol.* 138: 235–253.
- Cosart, T., A. Beja-Pereira, S. Chen, S. B. Ng, J. Shendure, and G. Luikart. 2011. Exome-wide DNA capture and next generation sequencing in domestic and wild species. *BMC Genomics* 12: 347.
- Cruaud, A., M. Gautier, M. Galan, J. Foucaud, L. Sauné, G. Genson, E. Dubois, S. Nidelet, T. Deuve, and J.-Y. Rasplus. 2014. Empirical assessment of RAD sequencing for interspecific phylogeny. *Mol. Biol. Evol.* 31: 1272–1274.
- Drew, R. A. I., G. H. S. Hooper, and M. A. Bateman. 1978. Economic fruit flies of the South Pacific Region. *Economic fruit flies of the South Pacific Region*.
- Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler, and S. E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6: e19379.
- Emerson, K. J., C. R. Merz, J. M. Catchen, P. A. Hohenlohe, W. A. Cresko, W. E. Bradshaw, and C. M. Holzapfel. 2010. Resolving postglacial phylogeography using high-throughput sequencing. *Proc. Natl. Acad. Sci.* 107: 16196–16200.
- Ferro, M. L., and J.-S. Park. 2013. Effect of propylene glycol concentration on mid-term DNA preservation of coleoptera. *Coleopterists Bull.* 67: 581–586.
- Hecht, B. C., N. R. Campbell, D. E. Holecck, and S. R. Narum. 2013. Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. *Mol. Ecol.* 22: 3061–3076.
- Hogan, F. E., R. Cooke, C. P. Burridge, and J. A. Norman. 2008. Optimizing the use of shed feathers for genetic analysis. *Mol. Ecol. Resour.* 8: 561–567.
- Hohenlohe, P. A., S. Bassham, P. D. Etter, N. Stiffler, E. A. Johnson, and W. A. Cresko. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genetics* 6: e1000862.
- Krosch, M. N., M. K. Schutze, K. F. Armstrong, Y. Boontop, L. M. Boykin, T. A. Chapman, A. Englezou, S. L. Cameron, and A. R. Clarke. 2013. Piecing together an integrative taxonomic puzzle: Microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis* sl (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. *Syst. Entomol.* 38: 2–13.
- Lozier, J. D. 2014. Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in North American bumble bees using RAD sequencing. *Mol. Ecol.* 23: 788–801.
- McCormack, J. E., S. M. Hird, A. J. Zellmer, B. C. Carstens, and R. T. Brumfield. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. *Mol. Phylogenet. Evol.* 66: 526–538.
- Michaud, C. L., and D. R. Foran. 2011. Simplified field preservation of tissues for subsequent DNA analyses. *J. Forensic Sci.* 56: 846–852.
- Moreau, C. S., B. D. Wray, J. E. Czekanski-Moir, and B. E. R. Rubin. 2013. DNA preservation: a test of commonly used preservatives for insects. *Invertebr. Syst.* 27: 81–86.
- Nadeau, N. J., S. H. Martin, K. M. Kozak, C. Salazar, K. K. Dasmahapatra, J. W. Davey, S. W. Baxter, M. L. Blaxter, J. Mallet, and C. D. Jiggins. 2013. Genome-wide patterns of divergence and gene flow across a butterfly radiation. *Mol. Ecol.* 22: 814–826.
- Nagy, Z. T. 2010. A hands-on overview of tissue preservation methods for molecular genetic analyses. *Org. Divers. Evol.* 10: 91–105.
- Payne, R. W. 2009. *GenStat*. Wiley Interdiscip. Rev. Comput. Stat. 1: 255–258.
- Prestrelski, S. J., N. Tedeschi, T. Arakawa, and J. F. Carpenter. 1993. Dehydration-induced conformational transitions in proteins and their inhibition by stabilizers. *Biophysical J.* 65: 661.
- Rizzo, J. M., and M. J. Buck. 2012. Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prev. Res.* 5: 887–900.
- Salvi, G., P. De Los Rios, and M. Vendruscolo. 2005. Effective interactions between chaotropic agents and proteins. *Prot. Struct. Funct. Bioinform.* 61: 492–499.
- Schutze, M., M. Krosch, K. Armstrong, T. Chapman, A. Englezou, A. Chomič, S. Cameron, D. Hailstones, and A. Clarke. 2012. Population structure of *Bactrocera dorsalis* ss, *B. papayae* and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: Evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. *BMC Evol. Biol.* 12: 130.
- Smith, L. M., and L. A. Burgoyne. 2004. Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecol.* 4: 4.

- Stevens, M. M., G. N. Warren, J. Mo, and D. I. Schlipalius. 2011. Maintaining DNA quality in stored-grain beetles caught in Lindgren funnel traps. *J. Stored Prod. Res.* 47: 69–75.
- Straub, S. C. K., M. Parks, K. Weitemier, M. Fishbein, R. C. Cronn, and A. Liston. 2012. Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics. *Am. J. Bot.* 99: 349–364.
- Vink, C. J., S. M. Thomas, P. Paquin, C. Y. Hayashi, and M. Hedin. 2005. The effects of preservatives and temperatures on arachnid DNA. *Invertebr. Syst.* 19: 99–104.
- Williams, S. T. 2007. Safe and legal shipment of tissue samples: does it affect DNA quality? *J. Molluscan Stud.* 73: 416–418.