Propylene glycol – a useful capture preservative for spiders for DNA barcoding

Hubert Höfer, Jonas Astrin, Joachim Holstein, Jörg Spelda, Franziska Meyer & Natalie Zarte

doi: 5431/aramit5005

Abstract. The usefulness of propylene glycol as capture preservative in pitfall traps, with the aim of using the captured spiders for DNA barcoding, was tested. For this purpose a laboratory experiment on the conserving and/or denaturing effect of propylene glycol on mitochondrial DNA (COI) was set up. For the experiment 110 specimens of the common and abundant wolf spider species *Pardosa lugubris* were manually captured, killed and incubated from one to four weeks in either pure or watered propylene glycol or 70 % denatured ethanol. Rates of successful sequencing, following a standard protocol, did not differ between samples incubated in propylene glycol and in the more commonly used ethanol. Thus, within four weeks, propylene glycol did not significantly denaturize mitochondrial DNA. In two field studies, pitfall traps with propylene glycol captured more spiders than traps with acetic acid. The effect was significant only in one of two field trials, but then consistent at three different sites and the three dominant spider families. Based on these results and our operating experience, we recommend propylene glycol as a capture preservative for (pitfall) traps to obtain specimens for DNA barcoding identification.

Keywords: acetic acid, capture efficiency, DNA preservation, GBOL, German Barcode of Life, methods, pitfall traps

Zusammenfassung. Propylenglykol eignet sich zum Fang von Spinnen für DNA-Barcoding. Die Eignung von Propylenglykol als Fangflüssigkeit in Bodenfallen zum Fang von Spinnen für eine Identifikation durch DNA-Barcoding wurde in einem Laborexperiment zur Konservierung bzw. Denaturierung mitochondrialer DNA getestet. Für das Laborexperiment wurden 110 Individuen der häufigen und abundanten Wolfspinnenart *Pardosa lugubris* gefangen und in purem oder verwässertem Propylenglykol oder 70 %igem denaturierten Äthanol getötet und zwischen ein und vier Wochen erstkonserviert. Die Verwendung von Propylenglykol statt Äthanol führte in keiner Variante zu einer signifikanten Senkung der Erfolgsrate für DNA-Sequenzierung nach einem Standardprotokoll. Es fand also innerhalb von 4 Wochen keine maßgebliche Denaturierung der mitochondrialen DNA in Propylenglykol statt. In zwei Feldstudien fingen Bodenfallen mit Propylenglykol als Fangflüssigkeit mehr Spinnen als mit Essig gefüllte Bodenfallen. Der Effekt war niedrig und nur in einer der beiden Feldstudien signifikant, aber konsistent in drei Teilflächen und den drei dominanten Spinnenfamilien. Aufgrund der Resultate empfehlen wir Propylenglykol als Fangflüssigkeit in (Boden-)Fallen, wenn Belege für DNA-Barcoding beschafft werden sollen, z.B. für die Erstellung einer Referenzdatenbank oder spätere (Nach-)Bestimmung.

DNA barcoding has proved to work well for species differentiation across most spider families (Barrett & Hebert 2005, Greenstone et al. 2005, Astrin et al. 2006, Hosseini et al. 2007, Blagoev et al. 2009, Robinson et al. 2009, Bayer & Schönhofer 2013, Miller et al. 2013). In Europe several national initiatives are currently underway to collect sequences for most species occurring in their countries. Based on good knowledge of the central European spider fauna and a sufficient number of interested arachnologists, fresh material for DNA barcoding can be easily sampled for a relatively high number of species by focused searching and manual sampling, followed by a thorough processing of the material – slice of a leg with a sterile blade or scissors, or using the whole body as source tissue – and DNA extraction following the standard protocol described in Ivanova et al. (2006). This covers the common and abundant species (e.g. *Araneus diadematus*, *Pisaura mirabilis*, *Pardosa palustris*), but even also many species with restricted distributions and/or abundances (e.g. *Titanoeca psammophila*, *Alopecosa striatipes*), due to the existing expert knowledge on occurrences (distribution, habitat preferences, phenology) on a local to regional scale. Joint efforts of various research groups and freelance arachnologists appear highly successful (demonstrated in the German Barcode of Life project, GBOL).

Most such initiatives rapidly arrived at rates of about 60 % of all species occurring in a specific

Hubert HÖFER, Franziska MEYER, Natalie ZARTE, Referat Zoologie, Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstr. 13, D-76133 Karlsruhe; E-mail: hubert.hoefer@smnk.de
Jonas ASTRIN, Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn; E-mail: J.Astrin.ZFMK@uni-bonn.de
Joachim HOLSTEIN, Staatliches Museum für Naturkunde Stuttgart, Rosenstein 1, D-70191 Stuttgart;
E-mail: holstein.smns@naturkundemuseum-bw.de
Jörg SPELDA, Sektion Arthropoda Varia, Zoologische Staatssammlung München, Münchhausenstraße 21, D-81247 München;
E-mail: spelda@zsm.mwn.de

submitted 12.1.2015, accepted 27.5.2015, online 29.6.2015

country/region that was successfully barcoded (examples listed at https://www.bolgermany.de/news-publikationen, accessed January 2015; Miller et al. 2013). However, finding fresh specimens of the remaining species becomes increasingly difficult. This is especially true for those spider species not readily identifiable in the field, e.g. many linyphiids, but also for rarely found species (e.g. *Clubiona kulczynski*), for which too few data are available to find/resample them intentionally. DNA barcodes for some species which are rare only on a regional/national level, will certainly be added to a cross-national (international) DNA barcode library by other countries, but not including the target country would result in knowledge gaps regarding genetic variability. One promising way to complete the DNA barcode library, including regional representatives, is to target species in museum collections (Miller et al. 2013). Although successful standard sequencing decreases with the age of the specimens – i.e. time since collected, duration of preservation in 70 % ethanol) from 90 % (fresh) to 60 % (10–20 years preserved) to 12.5 % (more than 50 years) and also with the size of the specimen – museum collections can contribute much to species completeness (Miller et al. 2013).

Notwithstanding all these efforts, DNA of some species and especially their genetic variability will only be provided by systematically collecting spider assemblages from all the different habitat types and bioregions. One important method to systematically sample spider assemblages are traps (e.g. pitfall traps, funnel traps/eclectors) and these usually use killing and preserving agents. Formerly widely used agents, like picric or acetic acid, formalin, saturated sodium chloride or specific mixtures like Galt's (5 % sodium chloride, 1 % saltpeter and 1 % chloral hydrate in water) unfortunately destroy/denature DNA (Vink et al. 2005, Stoeckle et al. 2010), whereas ethylene glycol (Gurdebeke & Maelfait 2002), propylene glycol (Vink et al. 2005), and also Renner solution (ethanol, glycerine, acetic acid, water) have shown to be suitable to preserve DNA (Stoeckle et al. 2010). Propylene glycol seemed to us the most promising substance due to its low toxicity to mammals (food additive) and its reasonable price (for the technical quality). We therefore tested propylene glycol (PG) for manageability in the field, capture and preserving ability, and lastly for its effect on DNA.

We wanted to test the usability of PG as capture agent in pitfall traps under simulated conditions (in the laboratory), using a large number of replicate samples (10 replicates per treatment, 30 replicates for PG versus ethanol, see Tab. 1), but also under real field conditions. After a non-systematic experiment using PG in pitfall traps and processing some of the captured spiders for barcode sequencing, we obtained an initial idea of success rates: for 74 % of specimens sampled in pitfall traps with propylene glycol (N = 367) COI sequences > 600 bp were obtained, in comparison to 92 % from recently hand collected and in ethanol (70 %) preserved material (N = 212).

Our initial hypothesis was that rain water would – at least in higher quantities – dilute the PG and would thus decrease DNA preservation. We wanted to check if the animals captured in PG during our normal field exposure times (two weeks) would still allow high success rates in Sanger-based DNA sequencing, whether small amounts of water entering the traps through rain or due to the hygroscopic nature of the preservative decrease the rate, and whether it is better to transfer the captured animals soon after collecting into ethanol instead of keeping them in PG until sorting and identification. Therefore we used incubation times of 1, 2 and 4 weeks for both preservatives. To imitate rain water intrusion in pitfall traps with PG as the capture liquid, we used treatment variants with 10 % and 50 % water; 10 % water was added in one variant from the beginning, in one variant 4 days after the spiders have been killed and conserved in pure PG. As we expected 50 % water to have a strongly negative effect, we did not test higher water contents.

Additionally, we wanted to check the capture efficiency of PG, i.e. whether PG had an (undesired) effect on the capture of spiders in pitfall traps, when compared to acetic acid, the preserving agent we had used in former studies based on pitfall traps. This was done in one field trial with 3 traps per agent in a relatively uniform habitat over 6 weeks and repeated in a second field trial with 3 traps per site and agent in three differing sites of a more heterogeneous habitat over 28 weeks. For both habitats we knew the spider fauna quite well from former studies.

Propylene glycol

From http://en.wikipedia.org/wiki/Propylene_glycol (accessed November 2014) the following information was extracted: Propylene glycol is a clear, colourless and hygroscopic liquid. It depresses the freezing point of water. In contrast to the closely related

Tab. 1: Experimental design of the test of DNA preservation. Abbreviations: Treat = treatment; 1. Pres. = first preservative, killing agent; Time 1 = incubation time in first preservative; 2. Pres. = second preservative; Time 2 = incubation time in second preservative; Transfer = Time until transfer to 96 % ethanol (not denatured); N = number of spider specimens; S = number of successful sequences (> 600 bp)

Treat	1. Pres.	Time 1	2. Pres.	Time 2	Transfer	N	S
P1	Propylene glycol	1 week			1 week	10	10
P2	Propylene glycol	2 weeks			2 weeks	10	10
P4	Propylene glycol	4 weeks			4 weeks	10	9
A1	Ethanol 70 %, denat.	1 week			1 week	10	10
A2	Ethanol 70 %, denat.	2 weeks			2 weeks	10	10
A4	Ethanol 70 %, denat.	4 weeks			4 weeks	10	10
PA	Propylene glycol	1 week	Ethanol 70 %, denat.	1 week	2 weeks	10	10
PW1	Propylene glycol, watered (10 % H_2O)	1 week			1 week	10	8
PW2	Propylene glycol, watered (10 % H_2O)	2 weeks			2 weeks	10	9
PWW1	Propylene glycol, watered (50 % H_2O)	1 week			1 week	10	9
PPW	Propylene glycol	3 days	Propylene glycol, watered (10 % H_2O)	4 days	1 week	10	10

ethylene glycol – which is very toxic to humans and many animals – propylene glycol is considered safe (GRAS) by the U.S. Food and Drug Administration, and it is used as an humectant (E1520), solvent, and preservative in food and for tobacco products and in many pharmaceuticals, including oral, injectable and topical formulations. Propylene glycol is an approved additive for dog food and considered safe for dogs, but not for cats. LD50 is 20 mL/kg for most laboratory animals. PG is readily degradable, without special risks in terrestrial environments, but high levels of biochemical oxygen demand during degradation in surface waters can adversely affect aquatic life by consuming oxygen needed by aquatic organisms for survival. Surface tension is about half of that of water (35.6 mN/m at 20°C). More information can be found under http://www.propylene-glycol.com/ (acessed November 2014).

Material and methods

We set up an experiment using manually collected specimens of *Pardosa lugubris* (Lycosidae) from one locality in Karlsruhe (20.6.2013), immediately transferring them into either propylene glycol, diluted propylene glycol, or 70 % denatured ethanol under the design summarized in Tab. 1. We used technical grade propylene glycol ($C_3H_8O_2$) from Herrlan PSM. All samples were always stored in a household refrigerator at the Staatliches Museum für Naturkunde Karlsruhe (SMNK) until finally cutting one leg of each spider and transferring it into absolute ethanol. These samples were then transported under cooled conditions to Stuttgart. DNA extraction was done in the laboratory of the Staatliches Museum für Naturkunde Stuttgart (SMNS) in March 2014 using the following procedure: Legs were used for parallelized, automated extraction in a Xiril Neon 100 robot, and amplification of the mitochondrial Cox1, 3' region (Simon et al. 1994) following the protocol developed by Ivanova et al. (2006). Unpurified PCR products were sent out to a sequencing facility (GATC GmbH, Konstanz). Contig assembly was handled using the software package Geneious (Biomatters, NZ) and lab data managed through a LIMS (Laboratory Information Management System). Specimens are deposited in the Arachnological collection of SMNK, DNA vouchers in the biobank of SMNS, field and laboratory data are linked through the database system Diversity Workbench (Triebel et al. 1999, Raub et al. 2012).

We compared the capture efficiency of pitfall traps with PG and acetic acid in two field trials:

In a meadow orchard on a village margin (Hohenwettersbach, Karlsruhe) from 17.4.-31.5.2012 (6

weeks): 3 traps with acetic acid, 3 traps with propylene glycol within appr. 100 m^2 (randomly selected);

In a dry/wet mountain heathland (Rasenbinsen-Bergheide at Schliffkopf, Northern Black Forest) from 18.4.-29.10.2012 (28 weeks): 9 traps with acetic acid and 9 traps with PG in an accidentally (April 2010) burned area (appr. 1 ha) originally covered with grass (3/3 traps) and pine (3/3 traps) and 3/3 traps in a neighbouring control area (not burned).

Pitfall traps were of the conventional type with plastic cups of 300 ml and an opening diameter of 67 mm, inserted in the ground, flush with the soil surface and filled with 100 ml preserving agent. Traps were protected against rain by non-transparent metallic roofs and to minimize capture of small vertebrates plastic funnels (opening diameter 1.6 cm, 30° angle) were inserted in the cups. Acetic acid was used in a 2.5 % solution with a drop of detergent added. Traps were emptied and refilled with fresh capture fluid every two weeks.

Statistics. Data from pitfall trapping were analyzed with a Manova (GLM) for the effects of fluid (capture agent) and site on the capture of individuals and species of all spiders, and the three dominant families with Statistica 9.0 (StatSoft Inc. 2009).

Results

All (100 %) 30 samples initially preserved in 70 % denatured ethanol and 93.75 % of 80 samples initially preserved in propylene glycol were successfully sequenced (i.e. COI sequence > 600 bp recovered). For only one of each treatment P4, PW2, PWW1 and two of PW1 or PCR was not successful, or the recovered sequence was < 300 (see Tab. 1).

Pitfall trapping in the meadow orchard during six weeks showed a positive, but not significant effect ($F_{1,34}$ = 0.08, p = 0.78) of PG (413 in PG versus 398 spiders in acetic acid) on the capture of spiders. The pitfall study of the spiders in the Black Forest showed a different picture (Tab. 2). Traps with propylene glycol captured more individuals and species at all three sites. The effect, although not high (1.1–1.4 ind./per trap, 0.4–0.6 species/trap) in size is highly significant (Manova: Wilks Lambda = 0.972, F(2, 233) = 3.34, p = 0.037) (Fig. 1) and consistent among families (Fig. 2) for the three most frequently captured families Agelenidae (*Inermocoelotes inermis*, *Coelotes terrestris*), Lycosidae (*Trochosa terricola*, *Alopecosa pulverulenta, Pardosa pullata, P. lugubris*) and Linyphiidae (*Glyphesis servulus*, *Pocadicnemis pumila*, *Micrargus herbigradus*) (most abundant species given).

Discussion

The results may indicate a possible negative effect of water intrusion (PW-treatments) into propylene glycol on DNA preservation, albeit without statistical significance. None of the treatments destroyed mitochondrial DNA (COI) at a rate which renders PCR amplification unsuccessful in a standard procedure for barcoding. This was partly expected based on the published literature (Gurdebeke & Maelfait 2002, Stoeckle et al. 2010). However, before recommending the practical application of propylene glycol as a capture fluid in traps, several aspects had to be checked. Fortunately, addition of small to medium water quantities – which are likely to occur in the field due to the hygroscopic nature of PG, but also rain – did not lead to DNA degradation critical for DNA barcode sequencing. However, strong water intrusion or longer storage in watered PG should probably be avoided to guarantee high success rates. Using PG in pitfall traps protected against rain, leaving them two weeks in the field and then transferring spiders to 70–80 % ethanol for morphological identification seems feasible to maintain the potential for a later use of specimens for DNA barcoding. The very high

Tab. 2: Capture efficiency of pitfall traps filled with acetic acid vs. propylene glycol at three mountain heathland sites in the northern Black Forest

	Acetic acid				Propylene glycol			
	individuals		species		individuals		Species	
Site	total	per trap	total	per trap	total	per trap	total	per trap
Pine, burned in 2010	80	2.4	22	2.2	109	3.6	27	2.9
Grass, burned in 2010	83	2.3	21	2.3	138	3.7	30	2.9
Control, not burned	130	3.5	23	3.0	178	4.6	37	3.4
total	293	2.7	41	4.9	459	4.2	51	6.8

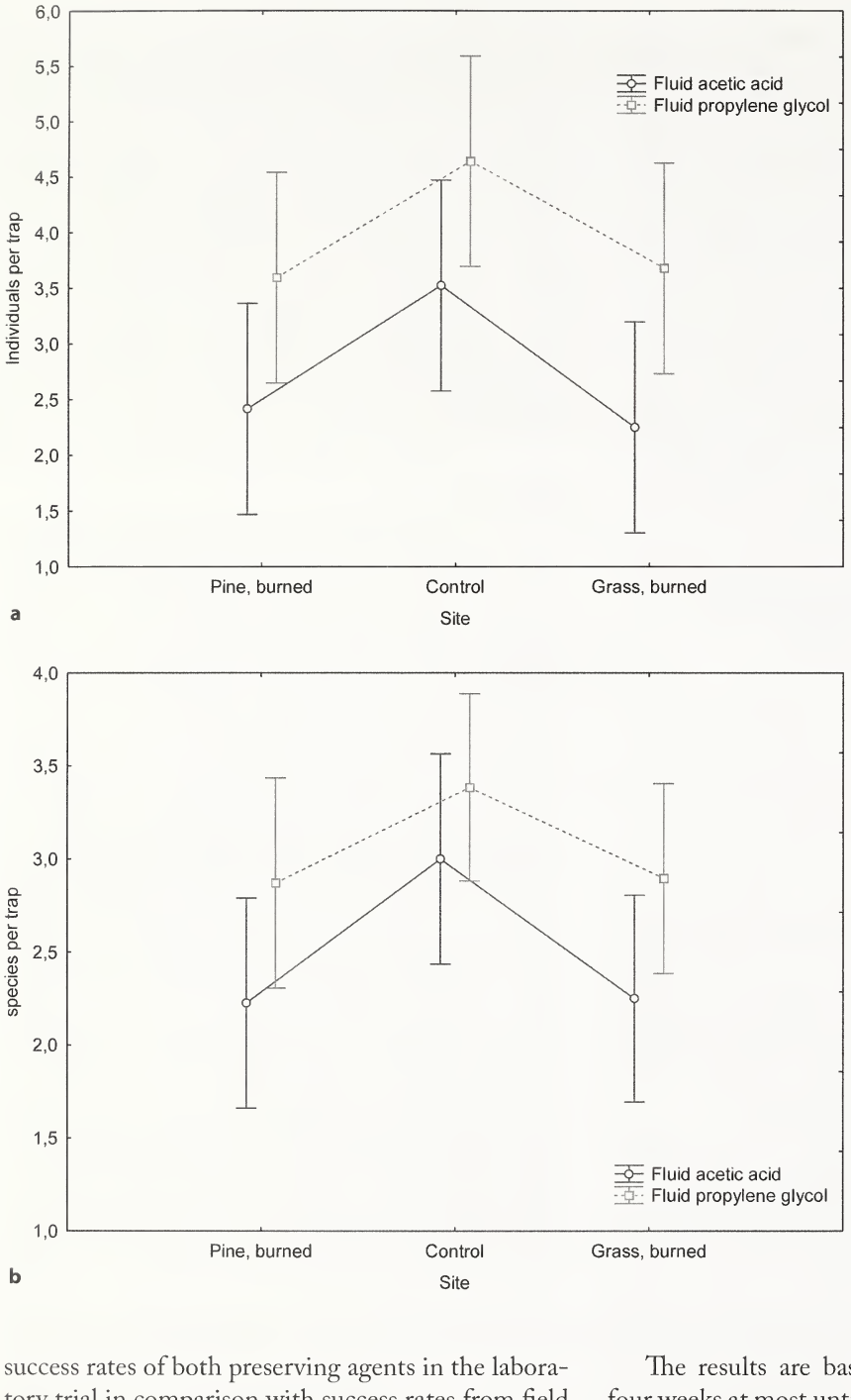

Fig. 1: Effect of the preservative (capture fluid) on the capture of individuals and species in pitfall traps at three sites in montane heathland in the northern Black Forest. Results of a two-way Manova (GLM): effect of fluid (Wilks Lambda = 0.972, F(2, 233) = 3.34, p = 0.037) and site (Wilks Lambda = 0.93420, F(4, 466) = 4.03, p = 0.003) on the two-week capture of **a.** individuals per trap; **b.** species per trap; vertical bars show 0.95 confidence intervals.

success rates of both preserving agents in the laboratory trial in comparison with success rates from field samples shows that in the field a series of unknown and uncontrollable factors further influence the preservation of the specimens. The yield of field samples could probably be increased by early (i.e. before transfer to denatured ethanol) cutting of a leg and its preservation in 96 % non-denatured ethanol under stable, cool conditions.

The results are based on short exposure times: four weeks at most until transfer into the recommended preservation fluid (non-denatured 96 % ethanol). However our focus lay on testing the use of a capture preservative under field conditions and realizing the usual processing of faunistic/ecological samples in our lab. The use of PG as a long-time preservation fluid in collections, as an alternative to the very expensive non-denatured ethanol, should be investiga-

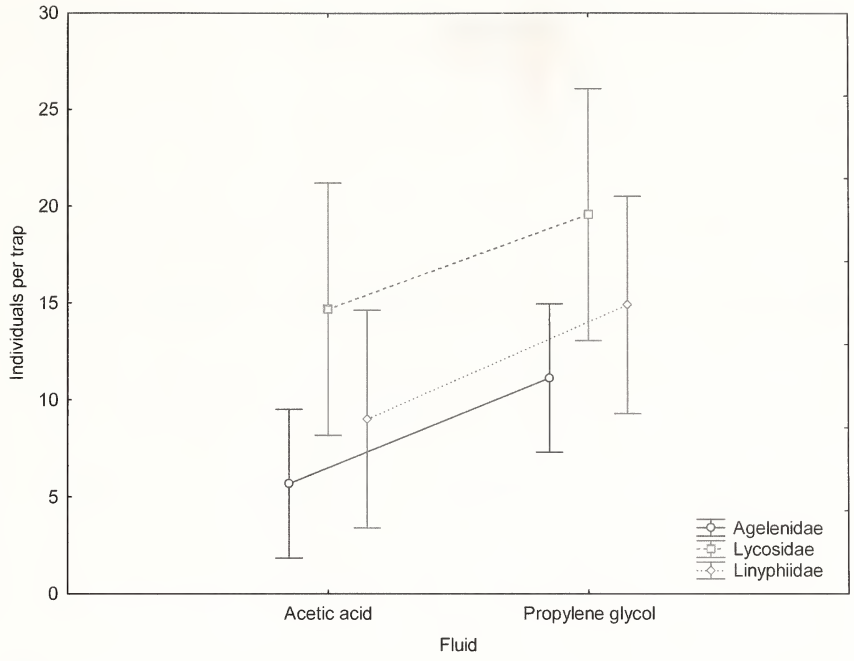

Fig. 2: Effect of the preservative (capture fluid) on the capture of the three dominant spider families in pitfall traps in montane heathland in the northern Black Forest. Results of a MANOVA (Wilks Lambda = 0.46, (F3, 14) = 5.34, p = 0.01); vertical bars show 0.95 confidence intervals.

ted over long-term experiments, including further aspects of long-term conservation. Vink et al. (2005) tested preservation in PG (and other preservatives) during six weeks and found PG to be superior to 95 % ethanol, at least for single copy genes. Regarding other aspects, the same authors reported a personal communication by M. J. Ramirez "It appears that propylene glycol may cause soft tissue shrinkage in specimens" (Vink et al. 2005).

In the light of the use of traps to capture spiders to complete the DNA barcode reference database of German species, we advocate the use of propylene glycol rather than acetic acid as a killing and preserving fluid in the field. Ethanol is not useful, due to its strong evaporation. The comparison of capture rates of PG with those of acetic acid shows that PG is more effective, perhaps due to its viscosity and lower surface tension compared to water. Although an attractiveness of particular capture liquids for individual species or groups has been shown and discussed by several authors (e.g. Adis & Kramer 1975, Adis 1979, Buchberger & Gerstmeier 1993, Gläser 2010), it was and is not feasible to compare even the more commonly used killing and preserving agents in their capture efficiency and undesirable effects in many habitats for the target taxa. However, we wanted to check at least how PG – as a promising agent under the new criteria of DNA preservation – behaves in this respect in comparison with the formerly used acetic acid. Most important here is the fact that PG was not selectively attractive to individual species or families and relative capture efficiency did not differ between the three sites. It can therefore be used to sample spider assemblages of different habitats, preserving the potential of identifying or verifying identifications (Vink et al. 2005, Blagoev et al. 2013) of trap sampled material through DNA barcoding, based on the comparison with a good reference database on German (European) spider species.

Acknowledgements

We thank Dr. Carlos Monje and Dr. Hong Shen for the extraction and processing of the DNA samples, M.Sc. Claudia Wesseloh for assistance in sorting the spiders and Dr. Steffen Bayer for commenting on an earlier version of the manuscript.

References

Adis J 1979 Problems of interpreting arthropod sampling with pitfall traps. – Zoologischer Anzeiger 202: 177-184

Adis J & Kramer E 1975 Formaldehyd-Lösung attrahiert *Carabus problematicus* (Coleoptera: Carabidae). – Entomologica Germanica 2: 121-125

Astrin JJ, Huber BA, Bernhard M & Klutsch CFC 2006 Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. – Zoologia Scripta 35: 441-457 – doi: 10.1111/j.1463-6409.2006.00239.x

Barret RDH & Hebert PDN 2005 Identifying spiders through DNA barcodes. – Canadian Journal of Zoology 83: 481-491 – doi: 10.1139/Z05-024

Bayer S & Schönhofer AL 2013 Phylogenetic relationships of the spider family Psechridae inferred from molecular data, with comments on the Lycosoidea (Arachnida: Araneae). – Invertebrate Systematics 27: 53-80 – doi: 10.1071/IS12017

Blagoev G, Hebert P, Adamowicz S & Robinson E 2009 Prospects for using DNA barcoding to identify spiders in species-rich genera. – ZooKeys 16: 27-46 – doi: 10.3897/zookeys.16.239

Blagoev G, Nikolova NI, Sobel CN, Hebert PDN & Adamowicz SJ 2013 Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. – BMC Ecology 13(44): 1-17 – doi: 10.1186/1472-6785-13-44

Buchberger P & Gerstmeier R 1993 Vergleich von Formol und Ethylenglykol als Fangflüssigkeiten in Barberfallen, unter besonderer Berücksichtigung der Carabidae. – Bericht der Naturforschenden Gesellschaft Augsburg 54: 77-93

Gläser B 2010 Methodischer Vergleich der Fangflüssigkeiten Benzoesäure, Ethylenglykol und Wasser an epigäischen Webspinnen (Arachnida: Araneae). Bachelorarbeit, Institut für Forstbotanik und Forstzoologie der TU Dresden, Tharandt. 43 pp.

Greenstone MH, Rowley DL, Heimbach U, Lundgren G, Pfannenstiel RS & Rehner SA 2005 Barcoding generalist predators by polymerase chain reaction: carabids and spiders. – Molecular Ecology 14: 3247-3266 – doi: 10.1111/j.1365-294X.2005.02628.x

Gurdebeke S & Maelfait J-P 2002 Pitfall trapping in population genetics studies: finding the right "solution". – Journal of Arachnology 30: 255-261 – doi: 10.1636/0161-8202(2002)030[0255:PTIPGS]2.0CO;2

Hosseini R, Keller MA, Schmidt O & Framenau VW 2007 Molecular identification of wolf spiders (Araneae: Lycosidae) by multiplex polymerase chain reaction. – Biological Control 40: 128-135 – doi: 10.1016/j.biocontrol.2006.10.007

Ivanova NV, DeWaard J & Hebert PDN 2006 An inexpensive, automation-friendly protocol for recovering high-quality DNA. – Molecular Ecology Notes 6: 998-1002 – doi: 10.1111/j.1471-8286.2006.01428.x

Miller JA, Beentjes KK, Helsdingen P van & IJland S 2013 Which specimens from a museum collection will yield DNA barcodes? A time series study of spiders in alcohol. – ZooKeys 365: 245-261 – doi: 10.3897/zookeys.365.5787

Raub F, Stierhof T & Höfer H 2012 Vom Karteikasten zu modernen Informationssystemen – die Entwicklung der zoologischen Datenbanken am Staatlichen Museum für Naturkunde Karlsruhe. – Carolinea 70: 91-101

Robinson EA, Blagoev GA, Hebert PDN & Adamowicz SJ 2009 Prospects for using DNA barcoding to identify spiders in species-rich genera. – ZooKeys 16: 27-46 – doi: 10.3897/zookeys.16.239

Simon C, Frati F, Beckenbach A, Crespi B, Liu H & Flook P 1994 Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – Annals of the Entomological Society of America 87: 651-701 – doi: 10.1093/aesa/87.6.651

StatSoft Inc. 2009 STATISTICA für Windows (Software-System für Datenanalyse). Version 9.0. – Internet: http://www.statsoft.com (12.1.2015)

Stoeckle BC, Dworschak K, Gossner MM & Kuehn R 2010 Influence of arthropod sampling solution on insect genotyping reliability. – Entomologia Experimentalis et Applicata 135: 217-223 – doi: 10.1111/j.1570-7458.2010.00977.x

Triebel D, Hagedorn G, Jablonski S & Rambold G (eds.) 1999 onwards. Diversity Workbench – A virtual research environment for building and accessing biodiversity and environmental data. – Internet: http://www.diversityworkbench.net (12.1.2015)

Vink CJ, Thomas SM, Paquin P, Hayashi CY & Hedin M 2005 The effects of preservatives and temperatures on arachnid DNA. – Invertebrate Systematics 19: 99-104 – doi: 10.1071/IS04039