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Review Article

Application of propylene glycol in DNA-based studies of invertebrates

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Abstract

High-throughput sequencing (HTS) studies on invertebrates commonly use ethanol as the main sample fixative (upon collection) and preservative (for storage and curation). However, alternative agents exists, which should not be automatically neglected when studies are newly designed. This review provides an overview of the application of propylene glycol (PG) in DNA-based studies of invertebrates, thus to stimulate an evidence-based discussion.

The use of PG in DNA-based studies of invertebrates is still limited (n = 79), but a steady increase has been visible since 2011. Most studies used PG as a fixative for passive trapping (73%) and performed Sanger sequencing (66%; e.g. DNA barcoding). More recently, HTS setups joined the field (11%). Terrestrial Coleoptera (30%) and Diptera (20%) were the most studied groups. Very often, information on the grade of PG used (75%) or storage conditions (duration, temperature) were lacking. This rendered direct comparisons of study results difficult, and highlight the need for further systematic studies on these subjects.

When compared to absolute ethanol, PG can be more widely and cheaply acquired (e.g. as an antifreeze, 13% of studies). It also enables longer trapping intervals, being especially relevant at remote or hard-to-reach places. Shipping of PG-conserved samples is regarded as risk-free and is authorised, pinpointing its potential for larger trapping programs or citizen science projects. Its property to retain flexibility of morphological characters as well as to lead to a reduced shrinkage effect was especially appraised by integrative study designs. Finally, the so far limited application of PG in the context of HTS showed promising results for short read amplicon sequencing and reduced representation methods. Knowledge of the influence of PG fixation and storage for long(er) read HTS setups is currently unavailable.

Given our review results and taking difficulties of direct methodological comparisons into account, future DNA-based studies of invertebrates should on a case-by-case basis critically scrutinise if the application of PG in their anticipated study design can be of benefit.

Key Words

alternative fixative, bioassessment, DNA integrity, DNA preservation, environmental monitoring

Introduction

DNA-based high-throughput sequencing (HTS) approaches such as DNA metabarcoding have lately revolutionised our ability to comparatively assess and monitor biodiversity over large geographical scales and at an unprecedented rate (Taberlet et al. 2012; Aylagas et al. 2016; Leese et al. 2018; Compson et al. 2020). Of particular relevance in practice are declining costs per sample, which are driven, among other things, by laboratory automatisation, comprehensive parallelisation of samples (i.e. multiplexing) and ever decreasing sequencing expenses (Leese et al. 2018). On the other hand, large-scale environmental programmes require a high number of appropriately conserved samples. Assuming that the number of samples to be processed within a scientific study or environmental program will continue to increase, also field costs per sample will account for an increasingly large financial share. It is therefore important to develop strategies to counterbalance the increasing costs of field

work, e.g. by reducing the time spent in the field, lowering hands-on times per sample by automatisation or adjusting collection and storage conditions (Holderegger et al. 2019; Ssymank et al. 2018).

The fixative (or trapping / killing agent) is a crucial parameter for DNA-based studies as it has to secure the integrity of the DNA from the very beginning (see e.g. Stein et al. 2013). Simultaneously, its costs are directly linked to the number of samples to be processed and to the volumes used. It is not unusual for larger studies to require several hundred to thousand litres of fixative. Liu et al. (2020) identified lab grade ethanol (>95%) to be the fixative of choice in the majority of DNA metabarcoding studies conducted between 2015 and 2019. Yet, lab grade ethanol is very expensive, has a high volatility, is not always easy to obtain and due to its flammability can cause problems during sample transportation and shipping. Alternative fixatives exist but are not widely applied, e.g. due to misunderstandings of chemical properties, tradition or simply lack of knowledge and availability (Weeks Jr and McIntyre 1997; Thomas 2008; Nagy 2010; Stoeckle et al. 2010; Gossner et al. 2016; Mahon et al. 2017).

As such an alternative, propylene glycol (or propane-1,2-diol; here further abbreviated as PG) has a number of characteristics that potentially render it beneficial in large-scale DNA-based HTS bioassessment and biomonitoring programmes: (i) low acquisition cost but high general availability, as it can be bought, for example, as low-budget antifreeze in specialist car dealers or as an additive from the cosmetic or food industry (additive E1520), (ii) non-toxicity (i.e. considered as a GRAS (generally regarded as safe) material), (iii) very low volatility, (iv) environmental safety, (v) risk-free transport of samples according to the regulations of the International Air Transport Association (IATA), and (vi) ability to ensure DNA integrity as well as to preserve most morphological characteristics (Thomas 2008; Nagy 2010).

Propylene glycol is a well-established agent in molecular cryobiology, notably used for the cryopreservation of sperms and cell cultures (Bank and Brockbank 1987; Hezavehei et al. 2018). For the study of invertebrates, it has been proposed as the chemical of choice in a standardised pitfall trap design for monitoring ground-active arthropod biodiversity (Brown and Matthews 2016; Hohbein and Conway 2018) and is applied as such within the North American National Ecological Observatory Network (NEON; Gibson et al. 2012; Hoekman et al. 2017) and the carabidologist community (Kotze et al. 2011). PG-based pitfall traps were also proposed as a standard and minimally disturbing method to investigate the subterranean fauna of the mesovoid shallow substratum (López and Oromí 2010; emptied every six months). Since 2001, PG fixation is applied by the Soybean Aphid Suction Trap Network (STN; Lagos-Kutz et al. 2020) for monitoring the 'aerobiological soup'. In the context of environmental genomic studies, Matos-Maraví et al. (2019) proposed PG as the fixative of choice for mass samples originating from pitfall traps and flight interceptions, but neither tests nor metadata had been published along with this statement.

The aim of this review is to summarise the findings of studies investigating the application of PG for DNA-based analyses of invertebrates. We will distinguish its application as a fixative (during sample collection) and as a preservative (for sample storage and curation). The collated information should help to transfer the available knowledge to the wider community, stimulating an evidence-based discussion on how to further reduce costs for DNA-conform sample collection and curation in larger environmental programmes by exploring alternative fixation and preservation agents.

Material and methods

Literature search

A topical core literature research was conducted on 07.10.2020 within the ISI Web of Science (WoS) and screening 'all databases'. The following search strings were investigated: (1) "propylene glycol" AND "DNA" AND "invertebrate*", (2) "propylene glycol" AND "DNA" AND "insect", (3) "propane-1,2-diol" AND "DNA" AND "invertebrate*" and (4) "propane-1,2-diol" AND "DNA" AND "insect". On the very same day, and because the WoS search only will detect literature records which are ISI-listed and only will retrieve hits in case the search string terms appear in the title, abstract, topic or as keywords, a complementing Google Scholar (GS) search was performed (as e.g. proposed by Piasecki et al. 2018). The GS search enables in-text searches and will also retrieve grey literature records such as field, laboratory and shipping protocols, pre-prints, theses and agency reports. The same four search strings were entered, selecting the 'exclude patents' option in GS. The keyword 'sperm*' was excluded in all searches as PG is frequently applied during the cryopreservation of sperms which would have resulted in a very high number of irrelevant hits. The key word 'invertebrate*' was excluded in the search strings (2) and (4) to not again screen insect literature records retrieved from (1) and (3).

The four search strings yielded the following number of literature records within the ISI WoS: (1) 20, (2) 8, (3) 0 and (4) 1. From those, only records which transparently stated the application of PG during specimen handling have been considered, resulting in a total of only 12 studies. Google Scholar search strings yielded the following constant number of literature records, regardless of whether the search was performed via two different IP-addresses in two countries (Luxembourg and Germany), within two different Google profiles, using a private browser window or being logged on/off from personal Google profiles: (1) 509, (2) 1930, (3) 23 and (4) 432.

Results

The majority of studies had to be excluded, a) because they used PG as a trapping material for invertebrates but only

cited literature referring to the application of DNA-based tools, or b) as they referred to yeast species molecularly analysed. Furthermore, reviews or studies which only dealt with the DNA analysis of e.g. parasites or gut contents of invertebrates were omitted, but will be discussed. Doctoral and master theses which were subsequently published as scientific articles were counted as a single entry.

A total of 79 publications was retrieved (Table 1, Fig. 1). Although the restricted number of literature records obtained exemplifies that the number of studies using PG as a fixative or preservative for subsequent molecular DNA-based analysis of invertebrates is not widely established (but an increase can be noted), still some patterns can be inferred. The analysed studies almost exclusively investigated terrestrial taxa, with Robinson et al. (2020; freshwater bulk sample) and Cordero et al. (2017; aquatic insects) being notable exceptions. The first studies using PG for DNA-based analysis of invertebrates originate from 2003 and 2005. Two of them focussed on honey bees (Rubink et al. 2003; Coulson et al. 2005) while Carter (2003) performed a comprehensive study on '*The effects of preservation and conservation treatments on the DNA of museum invertebrate fluid preserved collections*' using a terrestrial isopod species as reference. Those studies were accompanied by early publications on arachnids (Vink et al. 2005) and hemipterans (Scott et al. 2007). From today's perspective, studies focussing on Coleoptera (n = 24 studies, 30%) and Diptera (20%) dominated the dataset (Fig. 1). Although Diptera was the second largest of the individual groups retrieved, the first study was only published in 2012 and most of the records originate from the same team of authors.

In the majority of study designs, PG was used as a fixative for passive trapping (n = 53, 73%), and less frequently as a fixative upon manual collection of living specimens (32%). Either food-/laboratory-grade PG (14%; Sigma-Aldrich, Ajax FineChem, Neogen, Herrlan-PSM, Old World Industries, Better World Manufacturing, ClassiKool Ltd.) or PG-based antifreezes (13%; Lowtox, Absolute Zëro RV Waterline, Sierra, Uni-Gard) were used, but in 75% of all studies no further chemical properties or customer specifications were provided. PG concentrations were in most cases higher than 95% (for 70% of study designs), in fewer instances between 50–75% (16% study designs) or below 50% (11% study designs). Information on storage conditions for PG-preserved specimens was also very scarce: 27% of studies did not report any storage duration and for 41% of studies no storage temperatures were provided. Otherwise, PG-preserved specimens were stored for quite variable time spans, i.e. for more than half a year (10%), 1–6 months (10%), 1–4 weeks (20%), below 1 week (17%) or even for shipping only (17%, variable duration). If information was provided, specimens most often were stored at RT (28%), less frequently frozen (17%) or refrigerated (10%). Sanger sequencing was the most frequent evaluation method (66%), followed by PCR-based analyses (16%), HTS (11%) and microsatellite genotyping (9%). Studies performing two conceptual approaches, e.g. Sanger sequencing of COI and COI metabarcoding, were included in both categories.

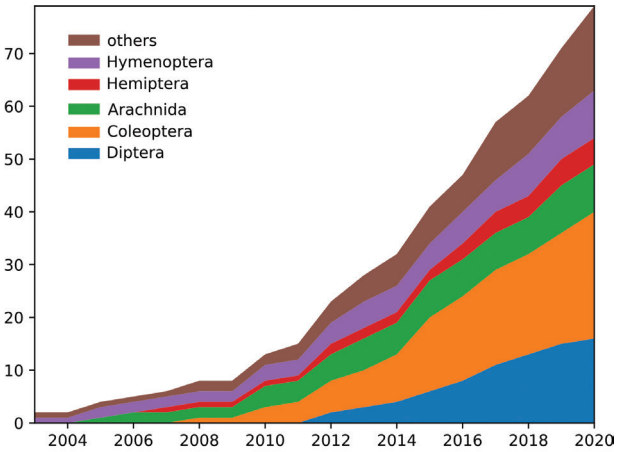

Figure 1. Cumulated number of studies which have used propylene glycol either as a fixative or preservative in the DNA-based analysis of invertebrates. Arranged by the taxonomic groups in focus. In cases where several of the indicated groups were targeted, the study was placed into the category "others". N = 79, as of 07.10.2020.

Discussion

Application of propylene glycol as a fixative

Our results indicate that PG is widely applied as a fixative in a variety of passive trapping methods (e.g. pan trap, funnel trap, aerial pitfall trap; baited and unbaited) and for various organism groups (mainly beetles and flies, but also spiders, bees and aphids). Likewise, actively collected single specimens or – in a few cases – invertebrate bulk samples were fixed with PG (e.g. Bowser et al. 2017, 2019; Cordero et al. 2017; Jusino et al. 2019; Robinson et al. 2020; Liu et al. 2020). This taxonomically widespread application mimics the use of PG in traditional non-DNA-based research and application domains (primarily pitfall trapping; see Hohbein and Conway 2018) now entering the molecular field. Research communities around aphids, spiders, carabids and tephritid fruit flies contributed most of the studies.

Amongst others, passive trapping intervals are determined by the accessibility of the sampling location, the volume of the trapping containers, the evaporation rate of the fixative and local environmental parameters such as humidity, temperature, rainfall or UV-exposure. In case of excessive heat and high temperatures (arid and hot), traps with ethanol or (salted) water frequently dry out and either have to be visited and re-filled more frequently, or short(er) trapping periods have to be chosen. The evaporation rate of PG is >500-times lower than for ethanol (Moreau et al. 2013). As such a low volatile agent, PG-equipped traps can retain their volumes more or less constant over several weeks to months (Hohbein and Conway 2018). However, long-term UV exposition may decompose PG into water, acetone and 2-propanol (i.e. isopropanol) (Nakahama et al. 2019). In interaction with its hygroscopic nature, even apparently large fixative volumes at the end of a long trapping period under summer conditions might contain a

Table 1. Overview of studies which have used propylene glycol either as a fixative or preservative for the DNA-based analysis of invertebrates. RT = room temperature; PG = propylene glycol; n.a. = not applicable or not available. Studies found by both the ISI Web of Science and Google Scholar searches, are marked in bold. All others were only detected by the latter search engine. N = 79, as of 07.10.2020.

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Carter (2003)	2003	Crustacea (Isopoda)	PCR-based visualisation (16S, 18S)	Propylene glycol	specimens directly placed in pure PG	12 months (RT)			double-stranded DNA profiles of PG-conserved specimens were sufficient for PCR, but likely not adequate for long-term storage of museum samples
Rubink et al. (2003)	2003	Hymenoptera (Apidae)	Microsatellite and CytB fragment analyses	Low-toxicity antifreeze (Lowtox, Prestone Inc., Danbury, CT)	specimens directly placed in pure PG	5, 20 or 90 days (20 °C or 40 °C)	95% ethanol	up to 4 months (4–6 °C)	nuclear and mtDNA were amplifiable even at the most extreme conditions (90 days, 40 °C), although a slightly decreasing trend was observed
					aerial pitfall trap with PG	3 weeks			
Coulson et al. (2005)	2005	Hymenoptera (Apidae)	CytB, 16S and COI fragment analysis	Propylene glycol	baited aerial pitfall trap with 50% PG and soap	ca. weekly for 8 months	95% ethanol	n.a.	well preserved for molecular analysis
Vink et al. (2005)	2005	Arachnida (Aranea, Scorpiones)	Actin and COI fragment analysis	Propylene glycol (99.5+% laboratory grade, Sigma-Aldrich)	specimens directly placed in pure PG	6 weeks (40 °C, 19–24 °C, 2–4 °C, -20 °C or -40 °C; dark)	95% ethanol (4 °C)	1 day	PG (and RNAlater) significantly better preserved nuclear and mtDNA than ethanol at various concentrations and in different study designs: mtDNA successfully amplified under most extreme conditions (6 weeks, 40 °C); ncDNA for small soft-bodied species only at room temperature or lower, and for large heavily sclerotized species at 2–4 °C or lower
Hendrixson (2006)	2006	Arachnida (Aranea)	Sanger sequencing of COI and 28S	Propylene glycol	pan trap with 1:1 PG and 100% ethanol	each 2 weeks for 2 months	100% ethanol, than 80% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Scott et al. (2007)	2007	Hemiptera (Aleyrodidae)	Sanger sequencing of COI	Propylene glycol	specimens directly placed in pure PG	n.a.			well preserved for morphological and molecular analyses
Gallego and Galián (2008)	2008	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	funnel trap with pure PG	1 week	absolute ethanol	n.a.	well preserved for morphological and molecular analyses, in particular in periods of no rainfall
Villacorta et al. (2008)	2008	Crustacea (Amphipoda)	Sanger sequencing of COI, COII and H3	Propylene glycol	baited pitfall trap with PG	several weeks to months	ethanol	n.a.	well preserved for morphological and molecular analyses
Castalanelli et al. (2010)	2010	Coleoptera	Sanger sequencing of mtDNA, single and multi-copy ncDNA genes	Propylene glycol	specimens directly placed in 20% PG	n.a.	ethanol	n.a.	very fast DNA isolation (ranging from 2–20 minutes); well preserved for parallel morphological and genetic analyses; specimens stored in 20% PG and ethanol did not differ from specimens stored in other preservatives
Horn (2010)	2010	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	pan trap with 75% PG	each two weeks	95% ethanol (refrigerator)	n.a.	specimens suitable for barcoding
Malumbres Olarte (2010)	2010	Arachnida (Aranea)	Sanger sequencing of COI	Mono-propylene glycol	pitfall trap with pure PG	2 weeks over 3 months	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Shoda-Kagaya et al. (2010)	2010	Coleoptera (Curculionidae)	Microsatellite genotyping	Propylene glycol	pheromone-baited trap with PG	n.a.	99.5% ethanol (-20 or 4 °C)	n.a.	specimens suitable for microsatellite analysis
Sonoda et al. (2010)	2010	Arachnida (Aranea)	Sanger sequencing of COI and restriction site analysis	Propylene glycol	pitfall trap with 20% PG	1 or 2 weeks	70% ethanol	months	well preserved for morphological and molecular analyses
Boyer et al. (2011)	2011	Annelida (Oligochaeta)	Sanger sequencing of COI and 16S	Propylene glycol	specimens directly placed in 98% ethanol	n.a.	PG	n.a.	well preserved for morphological and molecular analyses
Stevens et al. (2011)	2011	Coleoptera (Tenebrionidae, Bostrichidae)	Arginine kinase fragment analysis	99.5% Propylene glycol (Sigma-Aldrich Inc, St. Louis, MO)	specimens directly placed in PG (100%, 80%, 50% diluted or with PBS, with / without Triton-X 100)	3, 7 or 14 days (30 °C)	absolute ethanol (Sigma-Aldrich, molecular grade) (-80 °C)	n.a.	treatments with specimens stored in mixtures containing PG produced significantly less successful PCR results. PCR success was higher for specimens stored in pure PG than for 80% PG
				99.7% propylene glycol (Ajax FineChem Pty Ltd, Taren Point NSW, Australia)	Lindgren funnel trap with PG	1 week			specimens in PG produced significantly less successful PCR results than specimens maintained in PBS or dry
Castalanelli et al. (2012)	2012	Coleoptera (Dermestidae)	Sanger sequencing of COI, CytB and 18S	Propylene glycol	baited lure trap with 20% PG	two months	rinsed with sterile water, rinsed with 70% ethanol and stored in 95% ethanol	n.a. (-20 °C)	well preserved for morphological and molecular analyses

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Gibson et al. (2012)	2012	Coleoptera (Carabidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 66% PG	1 week	75% ethanol, or rinsed in water and stored in 95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Gruber et al. (2012)	2012	Hymenoptera (Formicidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 33% PG	1 day	95% ethanol (4 °C)	n.a.	well preserved for morphological and molecular analyses
Knee et al. (2012), Knee (2012)	2012	Arachnida (Acari)	Sanger sequencing of COI and 28S	Propylene glycol	baited Lindgren funnel trap with PG	~2 weeks	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Pelletier et al. (2012)	2012	Hemiptera (Aphidae)	Sanger sequencing of COI	Propylene glycol	trap with 50% PG with Bitrex and soap	each 2–3 days for 2 months	50% PG (4 °C)	up to 1 week	PG concentration checked in the field remained in the range 40–60%; collected material mostly suitable for morphological analysis, barcoding and RNA virus detection; although higher concentrations yielded better results
Renaud et al. (2012), Renaud (2012)	2012	Diptera (Muscidae)	Sanger sequencing of COI	Propylene glycol (food quality)	pan trap with 33% PG and soap	3–4 days	n.a.	n.a.	handling of PG was problematic because treated as hazardous waste and forbidden to dispose in local septic system; specimens suitable for barcoding and morphological analysis
Schutze et al. (2012)	2012	Diptera (Calliphoridae, Fanniidae, Muscidae, Tephritidae)	Sanger sequencing of COI	Propylene glycol	lure-baited hanging trap with pure PG	n.a.	assumably pure PG	n.a.	easy transport of samples; >90% of specimens morphologically characterised and barcoded
Vélez et al. (2012)	2012	Myriapoda (Chilopoda)	Sanger sequencing of COI and 16S	Propylene glycol	specimens directly placed in 95% or 75–80% ethanol	n.a.	PG, than 96% ethanol	n.a.	effective shipping of samples; well preserved for molecular analysis
Ferro and Park (2013)	2013	Coleoptera (Carabidae, Staphylinidae)	Sanger sequencing of COI	Propylene glycol (Neogen Corporation, Item No. 79231),	specimens directly placed in 100% ethanol	2 days	20%, 40%, 60%, 80% and pure PG (21 °C)	up to 6 months	positive PCR or sequencing results were obtained in all cases except for 20% PG
Krosch et al. (2013)	2013	Diptera (Tephritidae)	Microsatellite genotyping	Propylene glycol	insecticide-baited hanging trap with PG	n.a.			effective shipping of samples; well preserved for morphological and molecular analyses
Moreau et al. (2013)	2013	Hymenoptera (Formicidae)	Long-wavelength rhodopsin and COI fragment analysis	100% food-grade PG	specimens directly placed in pure PG	either remaining in PG for up to 10 months, or transferred into 95% ethanol after 6 months (6+4 months storage time)			PG and ethanol allowed for the highest PCR success rates. PG-preserved samples showed comparatively high DNA concentrations even after 10 months
Sikes and Stockbridge (2013), Stockbridge (2013)	2013	Mecoptera	Sanger sequencing of COII	Propylene glycol based antifreeze (Sierra brand)	pitfall trap with PG	two weeks	100% ethanol (-70F)	n.a.	well preserved for molecular analysis
Sim (2013)	2013	Arachnida (Aranea)	Sanger sequencing of COI, ITS and ND1	Propylene glycol	pitfall and pan trap with 50% PG	3–4 days for 2 weeks	95% ethanol (4 °C)	n.a.	well preserved for morphological and molecular analyses
Boykin et al. (2014)	2014	Diptera (Tephritidae)	Sanger sequencing of COI, NAD4–3', CAD, period, ITS1 and ITS2	Propylene glycol	insecticide-baited hanging trap with PG	variable	100% ethanol	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Endo et al. 2014	2014	Coleoptera (Carabidae); Myriapoda; Collembola	Sanger sequencing of COI, ITS1 and ANT	Propylene glycol	pitfall trap with PG:ethanol (1:1)	n.a.	100% molecular-grade ethanol	n.a.	well preserved for molecular analysis
Gómez (2014)	2014	Coleoptera (Carabidae)	Sanger sequencing of COI, CAD and 28S	Propylene glycol	pan trap with PG	n.a.			suitable for all molecular investigations; however, higher amplification success when PG-fixed specimens were dry pinned or transferred to 95% ethanol within 1–2 weeks
Chinvinijkul et al. (2015)	2015	Diptera (Tephritidae)	Sanger sequencing of ITS1	Propylene glycol	specimens directly placed in 95% ethanol	n.a.	pure PG	n.a.	effective shipping of samples; well preserved for molecular analysis
Fountain et al. (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI, CytB and ITS2	Propylene glycol	tarsal clips directly placed in PG	n.a.	95% ethanol (-20 °C)	n.a.	well preserved for molecular analysis
Haase and Zielske (2015)	2015	Gastropoda (Caenogastropoda)	Sanger sequencing of COI, 16S and ITS2	Propylene glycol	specimens directly placed in 70% ethanol	n.a.	PG, than 96% ethanol	PG-preservation only for shipping	effective shipping of samples; well preserved for morphological and molecular analyses
Höfer et al. (2015)	2015	Arachnida (Aranea)	Sanger sequencing of COI	Propylene glycol (technical grade, Herrlan-PSM)	specimens directly placed in pure, 90% or 50% PG	1, 2 and 4 weeks (refrigerator)	non-denatured 96% ethanol	n.a.	succesful barcoding under all conditions, but results potentially indicate a negative effect of water intrusion on PG-preserved specimens

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
					pitfall trap with PG, 2.5% acetic acid and detergent	2 weeks over 6 weeks	70–80% ethanol	n.a.	traps with PG captured more species (at three sites and for the most abundant families), but PG was not selectively attractive for particular taxa
Leblanc et al. (2015)	2015	Diptera (Tephritidae)	Sanger sequencing of COI	Propylene glycol (Better World Manufacturing, Fresno, CA)	baited Lindgren funnel trap with 25% PG	3–5 days	95% ethanol (freezer)	n.a.	well preserved for morphological and molecular analyses
Rugman-Jones et al. (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and 28S	Pink marine or recreational vehicle antifreeze (not for automobiles)	pheromone-baited Lindgren funnel trap with PG	weekly or bi-weekly	100% ethanol	n.a.	well preserved for molecular analysis; part of specimens collected in PG-based antifreeze according to official monitoring guidelines (Seybold et al. 2013)
Sánchez García et al. (2015), Sánchez García (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and COII	Propylene glycol	ethanol-baited funnal trap with PG	n.a.	n.a.	n.a.	well preserved for morphological and molecular analyses
Smith and Cognato (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and 28S	Propylene glycol	Lindgren funnel trap with PG	n.a.	100% ethanol	n.a.	well preserved for morphological and molecular analyses
Steininger et al. (2015)	2015	Coleoptera (Curculionidae)	Arginine kinase fragment analysis	99% extra pure PG (Fisher) and low-toxicity antifreeze (Lowtox, Prestone Inc.)	specimens directly placed in pure PG or PG-based antifreeze	2 or 7 days			very high qPCR success rates, no matter which storage conditions were used
Borges et al. (2016)	2016	Coleoptera (Zopheridae)	Sanger sequencing of COI, COII, tRNA-Leu gene and EF1α	Propylene glycol	pitfall trap with pure PG	n.a.	100% ethanol or acetone (refrigerator)	n.a.	well preserved for morphological and molecular analyses
Eigenbrode et al. (2016)	2016	Hemiptera (Aphidae)	Microsatellite genotyping	Propylene glycol	pan trap with PG	2-times a week for few months	95% ethanol	n.a.	well preserved for microsatellite analysis
Liu (2016)	2016	Diptera (Drosophilidae)	Microsatellite genotyping	100% food-grade propylene glycol	baited bottle trap with PG	1–2 days	95% ethanol (-60 °C)	n.a.	well preserved for morphological and microsatellite analyses
Patrick et al. (2016)	2016	Diptera (Calliphoridae, Fanniidae, Muscidae, Tephritidae)	High-quality genomic DNA for HTS approaches	99.5% propylene glycol (Sigma-Aldrich)	specimens directly placed in pure PG	1, 8, 13, 14 or 15 days (4 °C or -20 °C)			PG, 97–100% ethanol and AL buffer yielded high-quality genomic DNA, whereas RNA-free water, buffer AE and PBS failed. DNA concentration in ethanol was significantly higher at both temperatures
					lure-baited modified Steiner trap with PG	daily over few weeks	living specimens refrigerated than stored in pure PG (chilled)	20 days, afterwards for max. 1 month (-80 °C)	PG storage was chosen due to its higher overall practicability compared to ethanol and RNAlater; specimens were easily transported in airplane and provided high-genomic DNA for subsequent analyses
Postlethwaite (2016)	2016	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	pitfall trap with pure PG	days to few months	PG until pinning	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Robideau et al. (2016)	2016	Coleoptera (Scolytidae)	Real-time PCR of COI	Propylene glycol	Lindgren funnel trap with PG	n.a.	95% ethanol	n.a.	well preserved for morphological and molecular analyses
Wiseman et al. (2016)	2016	Coleoptera (Carabidae)	Sanger sequencing of COI, ITS2, 18S and 28S	Propylene glycol	pitfall trap with PG	n.a.	pinned and dried	n.a.	well preserved for molecular analysis
Boontop et al. (2017a), Boontop (2017)	2017	Diptera (Tephritidae)	Sanger sequencing of COI and microsatellite genotyping	Propylene glycol	specimens directly placed in pure PG	until shipping (RT)	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Boontop et al. (2017b), Boontop (2017)	2017	Diptera (Tephritidae)	Sanger sequencing of COI and microsatellite genotyping	Propylene glycol	specimens directly placed in pure PG	until shipping (RT)	95% ethanol (-20 °C)	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Bowser et al. (2017)	2017	Arthropod sweep net bulk sample	COI metabarcoding	Propylene glycol antifreeze (Uni-Gard -100)	specimens directly placed in pure PG	n.a. (-23 °C)	100% ethanol, rinsed with PBS prior to extraction	21 days	effective shipping of samples; well preserved for morphological and genomic analyses
Cordero et al. (2017)	2017	aquatic insects	Sanger sequencing of COI	Propylene glycol	specimens directly placed in 80% PG	n.a.	95% ethanol	n.a.	specimens suitable for barcoding
Greenslade et al. (2017)	2017	Collembola (Dicyrtomidae)	Sanger sequencing of COI	Mono-propylene glycol (antifreeze)	baited pitfall trap with PG and soap	2 days	Nesbitt solution	over night	well preserved for morphological and molecular analyses
Hoekman et al. (2017)	2017	Coleoptera (Carabidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 50% PG	each 2 weeks over entire growing season	95% ethanol, renewed after 24 h	up to several months	specimens highly suitable for morphological and genetic identification

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Langer et al. (2017)	2017	Diptera (Calliphoridae)	Sanger sequencing of COI	nontoxic recreational vehicle antifreeze	baited bottle trap with PG	n.a.	80% ethanol	n.a.	well preserved for morphological and molecular analyses
Lefort et al. (2017)	2017	Hemiptera (Aphidae)	COI metabarcoding	Propylene glycol	specimens directly placed in pure PG	n.a.	n.a. (-80 °C)	n.a.	effective transport of samples; DNA suitable for high-throughput sequencing applications
Perry et al. (2017)	2017	Lepidoptera (Plutellidae)	SNP assay (RAD-Seq)	Propylene glycol (USP grade)	specimens directly placed in pure PG	n.a. (-20 °C)			DNA suitable for population genomic SNP analysis
Ulyshen et al. (2017)	2017	Coleoptera (Lucanidae)	Sanger sequencing of COI	Propylene glycol	baited flight intercept trap with PG	each 2 weeks over 6 months	n.a.	n.a.	specimens well preserved for barcoding
Daglish et al. (2018)	2018	Coleoptera (Bostrichidae)	PCR-based resistance marker screening (*rph2*)	Propylene glycol	lure-baited Lindgren funnel trap with PG	n.a.	n.a.	n.a.	genomic DNA suitable for mass screening of resistance marker
Grando et al. (2018)	2018	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	Vane trap with PG	n.a.	70% ethanol	few months (10 °C)	well preserved for morphological and molecular analyses
Gregoire Taillefer and Wheeler (2018)	2018	Diptera	Sanger sequencing of COI	Propylene glycol	pan trap with 50% PG and soap	7–8 days	95% ethanol or air-dried or ethyl acetate or hexamethyl-disilazane	n.a.	well preserved for morphological and molecular analyses
Ide et al. (2018)	2018	Hymenoptera (Formicidae)	LAMP (loop-mediated isothermal amplification) assay	Propylene glycol	baited pan trap with pure PG	3 h	washed in 99.5% ethanol, air-dried (RT)	n.a.	well preserved for molecular analysis
Muturi et al. (2018)	2018	Diptera (Culicidae; gut content)	Sanger sequencing of COI and 16S metabarcoding	Propylene glycol	suction trap with 50% PG	weekly from May to October	95% ethanol (-20 °C)	n.a.	specimens well preserved for barcoding and microbial gut content analysis
Angelella et al. (2019)	2019	Hemiptera (Aphidae)	SNP assay	Propylene glycol	pitfall trap with 25% PG	weekly for 14 weeks	undiluted ethanol (-80 °C)	n.a.	DNA suitable for population genomic SNP analysis
Bowser et al. (2019)	2019	Arthropod sweep net bulk sample	COI metabarcoding	Propylene glycol antifreeze (Uni-Gard -100)	specimens directly placed in pure PG	n.a. (-23 °C)	100% ethanol, rinsed with PBS prior to extraction	1 week	well preserved for genomic analysis
DiGirolomo et al. (2019)	2019	Coleoptera (Buprestidae)	Sanger sequencing of COI	Propylene glycol	barrel with baited collection cups with PG	several months	n.a.	n.a.	specimens well preserved for morphological analysis, but barcoding was only partly successfull
Jusino et al. (2019)	2019	Arthropod pitfall trap bulk sample	COI metabarcoding	Propylene glycol	pitfall trap with pure PG	2–3 days	100% ethanol (RT, -20 °C)	n.a.	well preserved for morphological and genomic analyses
Krosch et al. (2019)	2019	Diptera (Tephritidae)	Sanger sequencing of COI and COII	Propylene glycol	specimens directly placed in pure PG	n.a. (RT)			effective shipping of samples; well preserved for morphological and molecular analyses
Landi et al. (2019)	2019	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	ethanol-baited Lindgren funnel trap with PG	n.a. (Nov-Feb)	n.a.	n.a.	well preserved for morphological and molecular analyses
Lienhard and Schäffer (2019)	2019	Arachnida (Acari)	PCR-based visualisation	Propylene glycol	specimens directly placed in pure PG	several weeks	absolute ethanol	1 day	results for DNA quality and quantity of PG-conserved specimens similar to ethanol-conserved specimens
Nakahama et al. (2019)	2019	Orthoptera (Gryllidae)	PCR-based visualisation (COI)	Propylene glycol	exposure to ethyl acetate vapour, followed by dehydration in 99.5% ethanol	1 h and 24 h	99% PG	1, 6 and 12 months	all replicates with PG-preserved (n = 12) were successfully amplified for all timepoints and three different fragment sizes
					-30 °C and dehydration in 99.5% ethanol	both 24 h			
					-30 °C	24 h			
Ramírez et al. (2019)	2019	Arachnida (Aranea)	Sanger sequencing of COI, 12S, 16S, H3, 18S and 28S	Propylene glycol (Sierra antifreeze)	pitfall trap with PG	~30 days	95% ethanol for transport; 100% ethanol storage	n.a.	well preserved for morphological and molecular analyses, although heavy rainfall diluted PG in traps and has led to sediment wash-in
Taillefer and Wheeler (2019)	2019	Diptera (diverse Schizophora families)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 50% PG, drop of detergent	6–8 days	95% ethanol	n.a.	well preserved for morphological and molecular analyses
Ballare et al. (2020)	2020	Hymenoptera (Anthophila)	ddRAD sequencing	Propylene glycol	Vane trap with PG, amongst other treatments (e.g. pan trapping with soapy water; netted specimens placed in ethanol)	5 days	100% ethanol	n.a. (until pinning)	all treatments produced a large number of high-quality loci (>4,000, ~20×). In comparison, the two PG-preserved species showed average DNA concentrations, but higher than average mean locus depths and lower than average mean numbers of polymorphic loci

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Krosch et al. (2020)	2020	Diptera (Tephritidae)	Sanger sequencing of COI	Propylene glycol	specimens directly placed in pure PG	n.a. (RT)			effective shipping of samples; suitable for DNA barcoding
Liu et al. (2020)	2020	Coleoptera (diverse)	COI and 16S metabarcoding	100% food-grade propylene glycol	pitfall trap with pure PG	30 days	95% ethanol	n.a. (-20 °C)	well preserved for morphological and molecular analyses
Mitchell et al. (2020)	2020	Coleoptera (Scarabaeidae)	Sanger sequencing of COI	Propylene glycol	flight intercept trap with PG:water mix	n.a.	ethanol (after 2–4 weeks)	n.a.	well preserved for morphological and molecular analyses
Moricca et al. (2020)	2020	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	lured Lindgren funnel trap with 30% PG	15 days	96% ethanol	n.a.	well preserved for morphological and molecular analyses, as well as for molecular screening of phytopathogenic fungi
Nakamura et al. (2020)	2020	Hymenoptera, Diptera, Coleoptera	PCR-based visualisation (COI)	Propylene glycol	specimens directly placed in 98% PG	2 weeks to 205/215 days (RT)			all, respectively, 96% of PG-preserved specimens produced PCR bands after 2 weeks and 6 months for two primer pairs and the DNeasy Blood and Tissue kit; performance was better than for 99.5% ethanol; PrepMan Ultra kit performance was less sophisticated
Robinson et al. (2020)	2020	benthic macroinvertebrate bulk sample	COI metabarcoding	Absolute Zëro RV Waterline Antifreeze (Recochem, Montreal, QC)	PG added to homogenized mock communities; or samples fixed in PG and than homogenized (ratio 1:3 fixative to sample)	3 days (RT)			evaporation step prior to DNA extraction was ommitted as PG does not inhibit PCR; communities are highly similar and even showed higher proportion of arthropod reads and higher richness than samples conserved with >99% lab grade ethanol

not to be underestimated amount of water and other compounds (Nagy 2010; Goetze and Jungbluth 2013; Borges et al. 2016), but chemical interactions and their effects on medium- to long-term DNA integrity are largely unknown. In addition to high temperatures, water dilution of PG in principle leads to a generally lower DNA integrity (Stevens et al. 2011; Höfer et al. 2015; Patrick et al. 2016; Nakamura et al. 2020), since water can lead to the hydrolysis of nucleic acids. Since most reviewed studies did not indicate the nature of the propylene glycol used, or field and storage temperatures, it was difficult to compare study outcomes. Nevertheless, in several studies, PG was used in the range of 20–80% as a trapping agent and traps deployed for a duration of 1–2 weeks. Specimens collected and fixed under these conditions were still sufficiently preserved for DNA analysis (Coulson et al. 2005; Sonoda et al. 2010; Hoekman et al. 2017; Angelella et al. 2019). In one of the most extreme cases, samples were fixed for 1 month in pitfall traps containing PG-antifreeze. The traps further experienced heavy rainfall and sediment wash-in. Nevertheless, Ramírez et al. (2019) successfully amplified six marker genes for the spider family under investigation and were able to morphologically investigate the specimens at hand.

Propylene glycol is in accordance with the Dangerous Goods Regulations of The International Air Transport Association (IATA). This means that PG-fixed samples are suitable for direct shipping and do not have to be transferred to another chemical agent on the spot. This characteristic was especially important for studies in remote areas (e.g. Schutze et al. 2012; Haase and Zielske 2015; Patrick et al. 2016; Bagnall 2016; Boontop et al. 2017a). In addition, it was seen as advantageous that PG can be relatively easily and cheaply obtained as an antifreeze in many parts of the world – a fact that does not always apply to absolute ethanol. As a major drawback, however, PG-based antifreezes might be regarded as special waste. As such, it can be prohibited to introduce them into local septic systems and the natural ground or to dispose them as domestic waste (Thomas 2008; Renaud 2012). Some national programmes might even prohibit the general use of antifreezes, as they can come along with additives such as lubricants, buffers, corrosion inhibitors and anti-foaming agents, whose impacts on natural environments are often not totally understood – or are considered carcinogenic (e.g. Tolytriazole, see Thomas 2008). Yet, much of the concern and regional bans relate to ethylene glycol-based antifreezes, which are toxic to humans and impose environmental risks. Readily available food-grade PG-based formulations such as from swimming pool or recreational vehicle antifreezes are generally regarded as safe (GRAS) material. They metabolize to lactic acid or substances of the Krebs cycle, which are natural metabolic products in the environment (Thomas 2008; Skvarla et al. 2014). Nevertheless, the waste-disposal issue imposed by using antifreeze fixatives (as it is true for larger quantities of ethanol as well) should be understood beforehand and appropriate measures taken.

Another relevant aspect refers to a potential catch bias caused by the fixative. Although Schmidt et al. (2006) have not integrated PG in their test, they showed that capture efficiencies of commonly used fixatives in pitfall trapping of spiders and carabids can greatly vary. Adding to this, Weeks Jr & McIntyre (1997), Calixto et al. (2007) and McCravy and Willand (2007) demonstrated that PG might not only affect the size of the sample but also its taxonomic composition. Höfer et al. (2015) reported that pitfall traps deployed with PG captured significantly more spider species, but were not selectively attractive for par-

ticular taxa. Nor was the pattern consistent across all sampling sites. Reliable reference data for PG capture rates still seem to be too small to make valid statements, but the community should be aware that such a taxonomic bias could exist. Additionally, mammals can be attracted by the use of PG, disturbing pitfall traps and biasing sampling designs (e.g. Aristophanous 2010 and own observations).

Application of propylene glycol as a preservative

Propylene glycol was only occasionally used as a medium-term preservative or storage medium. After PG fixation, most samples were stored in ≥95% ethanol until DNA extraction or PG-fixed samples directly analysed within a few days when retrieved from the field. Nevertheless, Nakamura et al. (2020) highlighted the potential use of PG as a chemical agent that can be applied from trapping to storage and for various taxonomic groups, so that hands-on times can be shortened as well as labour and equipment costs reduced (i.e. no specimen picking, circumventing transfer into another solution and container). The authors compared the COI-amplification rates of dipterans, hymenopterans and coleopterans preserved in 98% PG and 99.5% ethanol over a period of 2 weeks to >6 months at room temperature, concluding that DNA might be more long-term stable in PG than in ethanol. On the contrary, Patrick et al. (2016) tested various storage agents (including 99.5% PG and 97–100% ethanol) applicable in remote areas on three dipteran species under different temperatures. Although the experiment ended after 15 days, their results suggest that keeping PG fixed samples as cold as possible (e.g. packed in ice-filled boxes from hotel bars) is important to ensure short- to mid-term DNA integrity.

If we adopt the results to common practices of sample storage, it tells us that PG-preserved samples should preferably be stored cool and dark just like ethanol-preserved samples. Yet, DNA quality and quantity of long-term stored, chilled PG-preserved samples should be investigated in further detail.

Application of propylene glycol in HTS studies

Sufficiently high DNA quantities and DNA qualities are prerequisites in HTS studies. Lienhard and Schäffer (2019) evaluated DNA quality and quantity of ethanol- and PG-preserved oribatid mite species (<1 mm, preserved for several weeks), originating from seven DNA isolation methods suitable for high-throughput DNA sequencing. Although some study parameters had a significant effect on DNA quantity and quality, results for specimens preserved in PG or absolute ethanol generally suggest a high comparability. Similarly, Carter (2003) investigated the molecular weight spectra of double-stranded DNA (dsDNA) for specimens of the Rough Woodlouse *Porcellio scaber* preserved in PG, ethanol, ethyl acetate and 2-ethoxy ethanol for 12 months at room temperature. Ethanol- and cryo-preserved specimens provided the best quality and highest concentration of high molecular weight dsDNA for the investigated time period (with a remarkable drop after 24 months for ethanol). Yet, DNA quality (in terms of degradation) and DNA quantity (in terms of concentration) of specimens stored in PG were decreased, especially for longer fragments (>5 kbp).

However, specimens were stored at room temperature, which showed a strong degradation effect in Patrick et al. (2016) compared to samples which were kept cool.

Systematic studies analysing the impact of PG fixation and preservation in the context of HTS are widely lacking. Still, first case studies indicate a high applicability of PG fixation for short read amplicon sequencing (metabarcoding; Bowser et al. 2017, 2019; Lefort et al. 2017; Jusino et al. 2019; Liu et al. 2020; Robinson et al. 2020). In particular Robinson et al. (2020) tested the effect of PG antifreeze fixation of homogenised mock communities and benthic bulk samples. Their COI metabarcoding results indicate a generally high comparability of communities from ethanol and PG fixed samples. The latter even produced a higher proportion of arthropod reads and a higher richness of exact sequence variants (ESVs) when compared to ethanol samples.

Besides studies on short read amplicon sequencing, the applicability of PG was shown for short read reduced representation methods (RAD-sequencing; Perry et al. 2017; Angelella et al. 2019; Ballare et al. 2020). In particular, Ballare et al. (2020) conducted a comparative setting testing the impact of different field sample methods for SNP detection in wild bees, including PG-filled Vane traps. While a suitable DNA concentration and high locus depth were found for PG-fixed specimens, the number of loci recovered was comparatively low. Still, the two targeted wild bee species on average possessed more than 10,000 loci with a mean locus depth of >70. One potential cause for the lower than average mean number of polymorphic loci might be an incomplete PG evaporation, leading to an insufficient ethanol preservation prior to pinning. Alternatively, PG-stored samples were kept at room temperature, whereas other samples which showed higher numbers of polymorphic loci were stored at -20 °C.

To the best of our knowledge, no study used PG-fixed or -preserved specimens for invertebrate genome sequencing. Our assumption, however, would be that if samples are fixed and stored under optimal conditions, genome sequencing based on short read lengths should be possible. However, how ultra-long sequencing (e.g. Nanopore) will be affected by potential DNA degradation effects of PG remains unclear and should be explicitly addressed.

Integrative use cases of propylene glycol-conserved invertebrate samples

Besides the possibility to perform DNA-based analyses (e.g. microsatellite fingerprinting, DNA barcoding, metabarcoding and RAD-seq) directly on the invertebrates trapped or stored in PG, the samples seem suitable for a variety of research designs. Firstly, it has to be highlighted that PG-conserved specimens demonstrate a reduced shrinkage effect and specimens often remain appropriately conserved for morphological examina-

tions (Thomas 2008). More so, PG can be even added to ethanol-preserved specimens to retain flexibility and to reduce shrinkage effects without compromising DNA integrity and storage conditions (Boaze and Waller 1992; Carter 2003), e.g. for improving morphological determinability and dissection conditions (Karanovic et al. 2012, 2016; Martin 2016; Perina et al. 2018; Herrera Russert 2019). This 'relaxation' property of PG was exploited by several studies examining DNA markers and morphology of the very same specimens (Villacorta et al. 2008; Krosch et al. 2013; Boontop et al. 2017b; Gregoire Taillefer and Wheeler 2018; Grando et al. 2018). Furthermore, PG-conserved invertebrate samples were highly suitable for related integrative study designs, which are becoming increasingly popular in modern biodiversity studies. As such, Hu et al. (2017) investigated insect-associated microbiota by 16S rRNA amplicon sequencing of ants, Tremblay et al. (2018, 2019) screened phytopathogenic fungal propagules captured in insect traps via metagenomics, and Lynggaard et al. (2019) analysed bulk arthropod Malaise trap samples to detect vertebrates via 12S and 16S DNA metabarcoding. Molecular gut content analyses of PG-fixed invertebrate samples were e.g. performed for mosquitos (Muturi et al. 2018), fruit flies (Diepenbrock et al. 2018), carrion-feeding flies (Bagnall 2016) and diverse predatory groups (Murtiningsih 2014; Mabin et al. 2020). Endoparasites were investigated by e.g. Sokolova et al. (2010; microsporidians in booklice), Looney et al. (2012; horsehair worms in carabids and crickets), Barratt et al. (2012; braconid parasitoids in weevils) and Hartshorn et al. (2016; nematodes in wood wasps). Nie et al. (2011) detected the Potato virus Y from PG-fixed aphids via reverse transcription PCR. Finally, an addition of PG to pheromone/food traps might extend the lifetime of kairomones (Faleiro et al. 2016). Propylene glycol fixation of samples thus seems to be promising for a variety of integrative study designs.

Conclusions

There is currently only limited scientific literature on the use of PG for DNA-based analyses of invertebrates available, and even less so in the context of HTS. However, the investigated studies indicate that PG can be a versatile and worthwhile alternative for sample fixation (and potentially preservation) of various organism groups and in a range of methodological setups. Yet, generally valid statements about fixatives and preservatives are difficult to make (Nagy 2010; Short et al. 2018), and can be biased by comparing agents with contrasting water conditions affecting DNA integrity (see Nakamura et al. 2020), varying field vs. laboratory humidity conditions (due to the hygroscopic nature of PG) and storage temperatures or by confusing chemicals (e.g. Vaudo et al. 2018).

Future studies which plan the application of PG should critically scrutinize their trapping, specimen and storage conditions. For how many days are traps deployed? How will humidity, precipitation, UV exposure and temperature conditions in the sampling area affect the fixative? Do the targeted organisms allow for an easy tissue penetration by the fixative (e.g. soft-bodied vs. sclerotinised specimens)? Can PG reduce hands-on times and yet overall costs (e.g. no sample transfer for shipping; no evaporation prior to DNA isolation) (see e.g. Robinson et al. 2020)? In many cases, and in particular for new large-scale DNA-based monitoring programmes, one should not simply go by tradition but perform environment- and target group-specific tests and cost calculations before deciding upon the most suitable fixative.

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Supplementary material 1

Overview of DNA-based studies of invertebrates applying propylene glycol, sorted by year and taxonomic group

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Data type: study counts

Explanation note: Overview of DNA-based studies of invertebrates applying propylene glycol, sorted by year and taxonomic group.

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