Guidance on the collection of freshwater mussel (Bivalvia: Unionida) vouchers within Canada

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2022

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 3241

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Canadian Data Report of
Fisheries and Aquatic Sciences 3241

2022

Guidance on the collection of freshwater mussel (Bivalvia: Unionida) vouchers within Canada

by

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Cat. No. Fs97-4/3241E-PDFISBN 978-0-660-42389-0ISSN 1488-5387

Correct citation for this publication:

Morris, T.J., Martel, A.L., Wilson, G., Goguen, M.N., McNichols-O'Rourke, K.A. and Colm, J. E. 2022. Guidance on the collection of freshwater mussel (Bivalvia: Unionida) vouchers within Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 3241: v + 15 p.

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Collection of voucher specimens is an essential component of scientific investigation. Voucher specimens improve the integrity of collected data as the records can be independently and repeatably verified. Ideally a physical voucher will be collected for every species at every collection site however, limitations on processing time, storage capacity in the field and in archives, and permitting requirements when working with rare species may favour the collection of high quality digital vouchers in some circumstances. General guidance on the collection of physical and digital vouchers for freshwater mussels (Unionida) is provided and specific voucher requirements for all species found in Canada is provided. Examples of acceptable digital vouchers are also presented.

RÉSUMÉ

Morris, T.J., Martel, A.L., Wilson, G., Goguen, M.N., McNichols-O'Rourke, K.A. and Colm, J.E. 2022. Guidance on the collection of freshwater mussel (Bivalvia: Unionida) vouchers within Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 3241: v + 15 p.

La collecte de spécimens de référence est une composante essentielle de la recherche scientifique. Les spécimens de référence améliorent l'intégrité des données recueillies, car les observations peuvent être vérifiées de manière indépendante et répétée. Idéalement, on prélèvera un spécimen physique de référence pour chaque espèce et sur chaque site de collecte. Cependant, les limitations relatives au temps de traitement et à la capacité d'entreposage sur le terrain et dans les archives, ainsi que les exigences en matière de permis lorsque l'on travaille avec des espèces rares, peuvent favoriser la collecte de spécimens numériques de haute qualité dans certaines circonstances. Des directives générales sur la collecte de spécimens physiques et numériques pour les moules d'eau douce (Unionida) sont fournies, tout comme les exigences spécifiques en matière de spécimens pour toutes les espèces présentes au Canada. Des exemples de spécimens numériques acceptables sont également présentés.

INTRODUCTION

Collection of zoological voucher specimens has been a fundamental scientific activity since the mid 1700s (Lane 1996). Most often, specimens are kept during field surveys to confirm the identity of a species at a particular location, as some species cannot be reliably identified in the field and additional analyses, equipment, or expert validation is required. Vouchers are often used to understand a species' distribution (and changes to it), document morphometric variation in a species across its range, and catalogue biodiversity (e.g., Graf and Cummings 2011). A multitude of other research questions have benefitted from archived museum specimens for purposes beyond the original intent of collection. For example, stable isotope and microchemistry analyses from tissues (Verdegaal et al. 2005), and changes in morphology of collected specimens, have allowed scientists to understand the impacts of pollutants, climate change, and other environmental stressors over time (Rocha et al. 2014). Additionally, there can be consequences to not keeping voucher specimens (Wheeler 2003). If a species is taxonomically re-classified (e.g., *Villosa*, Watters 2018), collection records pre-dating the classification change that are not supported with a voucher are difficult or impossible to accurately update; a lack of vouchered specimens makes historical records questionable; as well, the diversity of species complexes can be missed without voucher specimens (e.g., Hoeh and Burch 1989).

Voucher specimens improve the integrity of collected data as the records can be independently and repeatably verified (Huber 1998, Wheeler 2003, Cummings and Bogan 2006). The gold standard of record keeping would be to always keep a physical specimen from each site. However, there is a trade-off with lab processing time, storage capacity in the field and in archives, and permitting requirements when working with rare species. Recent advances in conservation genetics and the development of tissue banks like the *National Biodiversity Cryobank of Canada* at the Canadian Museum of Nature (CMN) are making progress on issues around storage and processing and changing our thinking on voucher collection (Figure 1).

Across Canada, there is a lack of guidance on properly documenting freshwater mussels in aquatic surveys [see Mackie et al. (2008) for guidance in Ontario]. Many researchers have built capacity for specimen collections into their projects or have relationships with natural history institutions where specimens (empty shells, whole shell with tissue or small tissue fragments for genetic work) can be archived and curated. But depending on the nature of the survey and the practitioners conducting it, museum-quality specimens may not always be necessary or feasible. The purpose of this document is to provide guidance on collecting suitable physical vouchers and/or capturing appropriate digital vouchers of freshwater mussels that are of sufficient quality that the identity of a species can be verified. It is intended to be used by aquatic practitioners to improve data integrity and validity of records and may be useful for meeting permit requirements as well as ensuring sound conservation practices. This document covers all freshwater mussel species (Order Unionida) found in Canada. It builds on existing guidance for vouchering Ontario freshwater mussels from Mackie et al. (2008) and guidance for reporting collections found in Fisheries and Oceans Canada (DFO) and the Toronto Zoo's Clam Counter app for mobile devices (https://apps.apple.com/ca/app/clam-counter/id1221204178). It was developed in parallel with the *Voucher collection guidance for freshwater fishes in DFO's Ontario and Prairie and Arctic Regions* (Mandrak et al. 2022).

METHODS

CONSIDERATIONS

It is beyond the scope of this report to cover ethical considerations, permit-related conditions, or other legislative requirements involved in the collection of voucher specimens. It is assumed that the user of this report has obtained necessary training and permits, consulted with First Nations communities and local governments and will follow all guidelines set out by the Canadian Council on Animal Care (CCAC); Federal, Provincial, and Territorial fisheries regulations; Workplace Hazardous Materials Information System (WHMIS); and Transportation of Dangerous Goods.

FRESHWATER MUSSEL IDENTIFICATION

Accurate identification and recording of all species captured is essential. At least one field crew member should be trained in freshwater mussel identification (e.g., Ontario Freshwater Mussel Identification Workshop, ateliers d'identification du Ministère des Forêts, de la Faune, et des Parcs du Québec) and reference materials including field guides should be available in the field at all times. Fisheries and Oceans Canada and the Toronto Zoo have produced the *Clam Counter* app for mobile devices (iOS and Android) which can be readily used in the field for viewing species range distributions, key identification features, and for obtaining direction on digital voucher collection. Preliminary identifications should be conducted and recorded in the field, and vouchers (physical or digital) of every species caught at a site should be kept for confirmation of identifications. Ideally, two or three samples of each species representing different size classes would be vouchered while ensuring that there will be no significant impact on the collected population. Directions on types and procedures for voucher collection are outlined below.

PHYSICAL VOUCHERS

SHELLS

Most freshwater mussel species occurring in Canada can be readily identified using conchological characteristics. This fact, along with the observation that freshwater mussel shells persist after soft tissues have decayed, means that the collection of empty shells can be an effective means of vouchering. Freshwater mussel shells can be collected from predator piles (i.e., middens) or from depositional areas at a site. Collection of shell vouchers allows the examination of internal structures (e.g., pseudocardinal and lateral teeth) in addition to external shell characteristics. Examples of recently dead, fresh shells (i.e., remnant flesh, intact ligaments and bright periostracum) should be collected for each species at each site whenever possible and can be used as physical vouchers for live specimens along with their digital vouchers (see below). Caution should be employed when examining old and weathered shells (i.e., lacking tissue or ligaments, chalky, dull or absent periostracum) as erosion can obscure important identification features such as exterior shell markings (e.g., rays and pustules, beak sculpture) or internal characters (e.g., tooth structure, muscle scars). At a site, each presumed species should be collected in a separate bag labelled with the site code, geographic coordinates, waterbody, detailed site location description, date, collector and presumed species name. Whenever possible, voucher labels (on waterproof paper) should also include information about the general habitat characteristics of the collection site. In addition, the use of a log (fieldnote) book is highly recommended, where all pertinent information about each collection site is recorded in details. No special preservative is required in the field, however once back in the lab, shells can be cleaned with soap and water and labelled directly on the inside of the shell

with a permanent marker/india ink. Petroleum jelly can be applied to the shell to prevent desiccation and flaking of the periostracum and to improve visibility of key identification features. However, consideration should be given to the intended use of the voucher as the application of preservatives may hinder future analytical opportunities (Morton 2006).

GENETIC SWABS

Several species are very difficult to morphologically distinguish from closely related species (see Table 1) while others may produce hybrids within certain portions of their geographic ranges (Porto-Hannes et al. 2021). Under these circumstances it may be necessary to use genetic techniques (e.g., barcoding, sequencing) to confirm identification. *Non-destructive Tissue Biopsy* or *Whole-animal (Live Shell) Collections* (see below) can be used for genetic identification when working with common species and when approved by federal/provincial permitting agencies. When dealing with a species at risk it is recommended that foot/visceral mass swabs be used to collect the sample in a non-destructive manner (Henley et al. 2006).

NON-DESTRUCTIVE TISSUE BIOPSY

Species identification may also be confirmed genetically through the collection of small tissue samples (< 1 cm^2) from the mantle (Berg et al. 1995). Tissue biopsies provide a greater amount of DNA than genetic swabs allowing for multiple extractions from a single collection and a lower rate of failed amplification without the need to sacrifice individuals. When collecting biopsy samples, reversing pliers can be used to gently separate the valves (Figure 2). Care should be taken to avoid damage to the adductor muscles caused by forcing too large a gape (Figure 2). Non-destructive tissue biopsy samples are ideal for long term storage in facilities such as the National Biodiversity Cryobank of Canada.

WHOLE-ANIMAL (LIVE SHELL) COLLECTIONS

Occasionally it is necessary to collect a whole animal (see Table 1). Juveniles (< 30 mm) can be placed directly into a preservative (e.g., 95% ethanol) whereas adults will need to be relaxed prior to placing them in the preservative. Relaxation can be achieved by cutting the anterior and posterior adductor muscles with a small sharp knife or scalpel – note that this procedure must be carefully done to ensure that the shell margin is not damaged by the knife or scalpel during the process. Once relaxed, the shell will gape and preservative will easily enter the body cavity preventing decomposition of internal tissues. In all cases, ensure that the specimen is fully covered in preservative, remove excess air and place a label written in pencil or indelible ink on waterproof paper that includes site code, geographic coordinates, waterbody, date, collector and presumed species name.

When a collection represents a significant range extension (i.e., first collection from a watershed) it is recommended to collect a whole specimen when not prohibited by permits.

DIGITAL VOUCHERS

Digital (photographic) vouchers are an accepted method of vouchering for many species (see Table 1) and may preclude the need to take physical vouchers if done correctly. The following guidance for taking digital vouchers is modified from Mackie et al. (2008). The collection of digital vouchers typically requires more time, equipment and skill than the collection of physical vouchers but results in less potential harm to individuals which can be quite important when working with sensitive species. A quality camera with macro photography capability is recommended though most modern mobile devices are now capable of taking adequate photographic vouchers. It is recommended that a camera stand or tripod/copy stand (Figure 3)

be used to ensure stability and consistency of digital vouchers. Whenever possible, photos should be taken in daylight rather than using artificial flash to ensure true colour representation. The photographer must be familiar with freshwater mussel identification and know the important characteristics of the species being photographed. Photos must be checked in the field to verify that image quality is sufficient (e.g., in focus) to identify the specimen and that the important identification features (Table 1) are clearly visible. Always include a scale bar in frame when collecting digital vouchers.

Three core shots of each specimen are required (Figure 4). A lateral view of the left valve will show the basic outline, the position of the beak, the presence/absence of a dorsal wing, the periostracum colour and markings (e.g., rays) as well as the presence/absence and location of any shell sculpture (e.g., pustules and ridges). The wide dorsal view will permit an assessment of the degree of inflation of the specimen. A dorsal close-up view of the beak will permit an assessment of the presence/absence and composition of the beak sculpture. Additional photographs may be required depending upon the putative field identification (Table 1). For all photographs it is essential that the camera remain in the correct orientation throughout to avoid distortion. Avoid taking photographs at oblique angles where physical features become distorted. Placing the specimen on a bed of modelling clay or sand will greatly assist with maintaining the proper orientation (Willsie et al. 2020).

Table 1 provides guidance on whether a digital voucher is acceptable for a species and, if so, what additional features need to be photographed. In some cases, the necessity of a physical voucher over a digital voucher varies regionally. Regardless of the type of photograph you are taking, it is imperative that the camera be zoomed in to ensure that the distinguishing characteristic fills the entire frame of the viewer and that a reference to scale exists in the photograph (i.e., a ruler or scale bar). The photograph numbers should be recorded next to the data sheet entry for that individual. It is good practice to include a photograph of the field sheet with the relevant site data (field number, waterbody name, geographic coordinates, date, collectors) at the start of the series of digital vouchers for each site. A label with the relevant site data could also be placed in the frame of the photograph. There should be only one specimen visible per photograph.

ARCHIVING VOUCHERS

All vouchers should be verified by at least one taxonomic expert, and their name and the date of verification should be recorded. Quality preserved vouchers should be archived with a museum or university collection where feasible (Figure 5).

Digital vouchers should be saved and the photographs labeled with the unique site number and photograph number at a minimum (preliminary species identity and reference to location recommended). They should be backed-up in a second location. Digital vouchers must be kept at least until they have been verified and all permitting submissions have been approved. Archiving digital vouchers on an external hard-drive (preferably a solid-state drive) is a low cost, space-saving solution for long-term storage. Most natural history museums have a collection database system that can archive digital images with the specimen records.

BACK-CORRECTION OF MISIDENTIFIED SPECIMENS

On occasion, specimens are misidentified in the field, and re-identified and verified in the laboratory from a physical or digital voucher as a different species. If all of the captured specimens were vouchered, the species identity(ies) can be easily updated for the entire catch. However, if many individuals were caught but only a single voucher kept, the identity of the other individuals cannot be ascertained. In studies where only presence/absence of a species is needed, only the species for which a voucher was kept should remain. If catch per unit effort or relative abundance are needed, the specimens for which there is no voucher should be up-listed in the record to genus or family (depending on the number of similar or closely related species).

ACKNOWLEDGEMENTS

We thank Jean-Marc Gagnon at the Invertebrate Collections at the Canadian Museum of Nature and Jessica Epp-Martindale of Fisheries and Oceans Canada for their valuable comments and suggestions on the manuscript. We are also in debt to Kathy Klein for valuable editorial comments on an earlier version of the text.

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Table 1. Guidance on voucher collection (preserved specimen vs. digital) for all Canadian Unionida. The known provincial/territorial distribution is indicated for each species. If collecting a species outside the range identified here a whole-animal preserved specimen should always be collected. When collecting digital vouchers it is recommended to also collect recently dead, fresh shells of each species identified at each site (if present) to allow confirmation of identification using internal shell characters. When permitting conditions differ from the information contained here the permit requirements are to be followed. Nomenclature follows Molluscabase eds. (2022).

Common Name	Scientific Name	BC	AB	SK	MB	ON	QC	NB	NS	NF	PEI	YK	NWT	NU	Preserved Specimen Essential	Core Photo Vouchers Required	Additional Photo Vouchers
Dwarf Wedgemussel	*Alasmidonta heterodon*							x							Genetic swab	Yes	
Elktoe	*Alasmidonta marginata*					x	x								No	Yes	Dorsal slope
Triangle Floater	*Alasmidonta undulata*					x	x	x	x						No	Yes	Heavy sculpture extending ventrally from beak
Brook Floater	*Alasmidonta varicosa*							x	x						No	Yes	Orange foot
Slippershell Mussel	*Alasmidonta viridis*					x									No	Yes	
Threeridge	*Amblema plicata*			x	x	x									No	Yes	Strongly developed ridges
Western Floater	*Anodonta kennerlyi*	x	x												No	Yes	
Winged Floater	*Anodonta nuttalliana*	x													No	Yes	Dorsal wing
Oregon Floater	*Anodonta oregonensis*	x													No	Yes	
Cylindrical Papershell	*Anodontoides ferussacianus*				x	x	x								No	Yes	
Tidewater Mucket	*Atlanticoncha ochracea*							x	x						No	Yes	
Rainbow	*Cambarunio iris*					x									No	Yes	
Pimpleback	*Cyclonaias pustulosa*					x									No	Yes	Broad green ray on beak, lack of pustules on beak
Purple Wartyback	*Cyclonaias tuberculata*					x									No	Yes	Pustules extending onto beak; pustules coalescing into ridges on posterior wing
Eastern Elliptio	*Elliptio complanata*					x	x	x	x						No	Yes	Pronounced posterior ridge
Elephantear	*Elliptio crassidens*						x								Whole animal	No	Hinge area
Northern Riffleshell	*Epioblasma rangiana*					x									No	Yes	
Snuffbox	*Epioblasma triquetra*					x									No	Yes	
Spike	*Eurynia dilatata*					x	x								No	Yes	

Common Name	Scientific Name	BC	AB	SK	MB	ON	QC	NB	NS	NF	PEI	YK	NWT	NU	Preserved Specimen Essential	Core Photo Vouchers Required	Additional Photo Vouchers
Wabash Pigtoe	*Fusconaia flava*				x	x									No	Yes	
Rocky Mountain Ridged Mussel	*Gonidea angulata*	x													No	Yes	Strong angular posterior ridge
Plain Pocketbook	*Lampsilis cardium*			x	x	x	x								No	Yes	Beak morphology, details of beak sculpture
Yellow Lampmussel	*Lampsilis cariosa*							x	x						No	Yes	
Wavyrayed Lampmussel	*Lampsilis fasciola*					x									No	Yes	
Eastern Lampmussel	*Lampsilis radiata*					x	x	x	x						Whole animal (Lake Ontario and St. Lawrence drainages)	Yes, if whole animal taken	
Fatmucket	*Lampsilis siliquoidea*		x	x	x	x	x						x	x	Whole animal (Lake Ontario and St. Lawrence drainages)	Yes, if whole animal taken	Beak morphology, details of beak sculpture
White Heelsplitter	*Lasmigona complanata*		x	x	x	x									No	Yes	Prominent dorsal wing
Creek Heelsplitter	*Lasmigona compressa*			x	x	x	x								No	Yes	
Flutedshell	*Lasmigona costata*				x	x	x								No	Yes	Fluting along posterior ridge
Black Sandshell	*Ligumia recta*			x	x	x	x								No	Yes	
Western Pearlshell	*Margaritifera falcata*	x													No	Yes	
Eastern Pearlshell	*Margaritifera margaritifera*						x	x	x	x	x				No	Yes	
Threehorn Wartyback	*Obliquaria reflexa*					x									No	Yes	
Hickorynut	*Obovaria olivaria*					x	x								No	Yes	Ensure a good lateral view of the shell showing position of beak
Round Hickorynut	*Obovaria subrotunda*					x									No	Yes	

Common Name	Scientific Name	BC	AB	SK	MB	ON	QC	NB	NS	NF	PEI	YK	NWT	NU	Preserved Specimen Essential	Core Photo Vouchers Required	Additional Photo Vouchers
Mucket	*Ortmanniana ligamentina*					x	x								No	Yes	
Rayed Bean	*Paetulunio fabalis*					x									No	Yes	
Round Pigtoe	*Pleurobema sintoxia*					x									Genetic swab (outside of the Sydenham R., Thames R., Grand R., or St. Clair delta)	Yes	
Pink Heelsplitter	*Potamilus alatus*				x	x	x								No	Yes	
Fragile Papershell	*Potamilus fragilis*					x	x								No	Yes	
Kidneyshell	*Ptychobranchus fasciolaris*					x									No	Yes	
Eastern Floater	*Pyganodon cataracta*					x	x	x	x		x				Whole animal (in Ontario)	Yes	
Newfoundland Floater	*Pyganodon fragilis*							x	x	x					No	Yes	
Giant Floater	*Pyganodon grandis*		x	x	x	x	x					x	x		No	Yes	
Lake Floater	*Pyganodon lacustris*					x									Whole animal	No	
Mapleleaf	*Quadrula quadrula*				x	x									No	Yes	Pustules extending onto beak
Eastern Pondmussel	*Sagittunio nasutus*					x									No	Yes	
Salamander Mussel	*Simpsonaias ambigua*					x									No	Yes	
Yukon Floater	*Sinanodonta beringiana*	x										x			Yes if collected in BC	Yes	
Creeper	*Strophitus undulatus*			x	x	x	x	x	x						No	Yes	
Lilliput	*Toxolasma parvum*					x									No	Yes	
Fawnsfoot	*Truncilla donaciformis*					x									No	Yes	
Deertoe	*Truncilla truncata*					x									No	Yes	
Paper Pondshell	*Utterbackia imbecillis*					x									No	Yes	
Alewife Floater	*Utterbackiana implicata*						x	x	x						No	Yes	

Figure 1. Some of the installations at the National Biodiversity Cryobank of Canada located within the Natural Heritage Building of the Canadian Museum of Nature, on 1740, Pink Road, Gatineau, Québec. This is where tissue samples, including mantle biopsies, of freshwater mussels can be stored in liquid nitrogen (-185°C) for curation and archiving for posterity. Photo credit Canadian Museum of Nature.

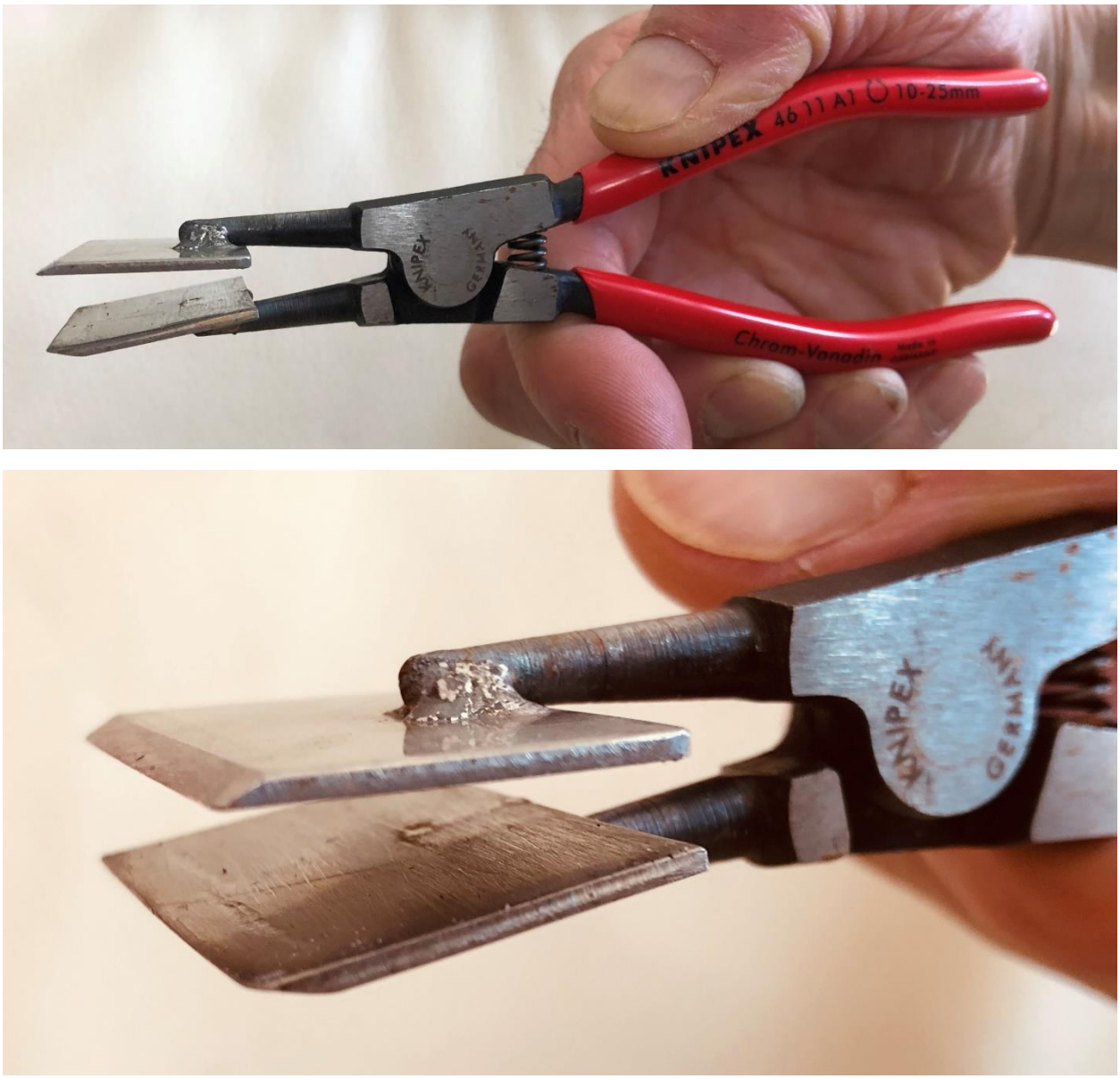

Figure 2. Pliers specially modified to gently open the valves of live unionid mussels to allow the collecting of DNA samples from the soft tissue of the mollusc. Note that the prying must be done carefully and slowly to avoid damaging the valves' edges. This tool can greatly facilitate the non-lethal collecting of DNA samples, including genetic swabs (foot or visceral mass), haemolymph (using a long-needled syringe), or non-destructive mantle biopsies (using fine dissecting scissors or scalpels).

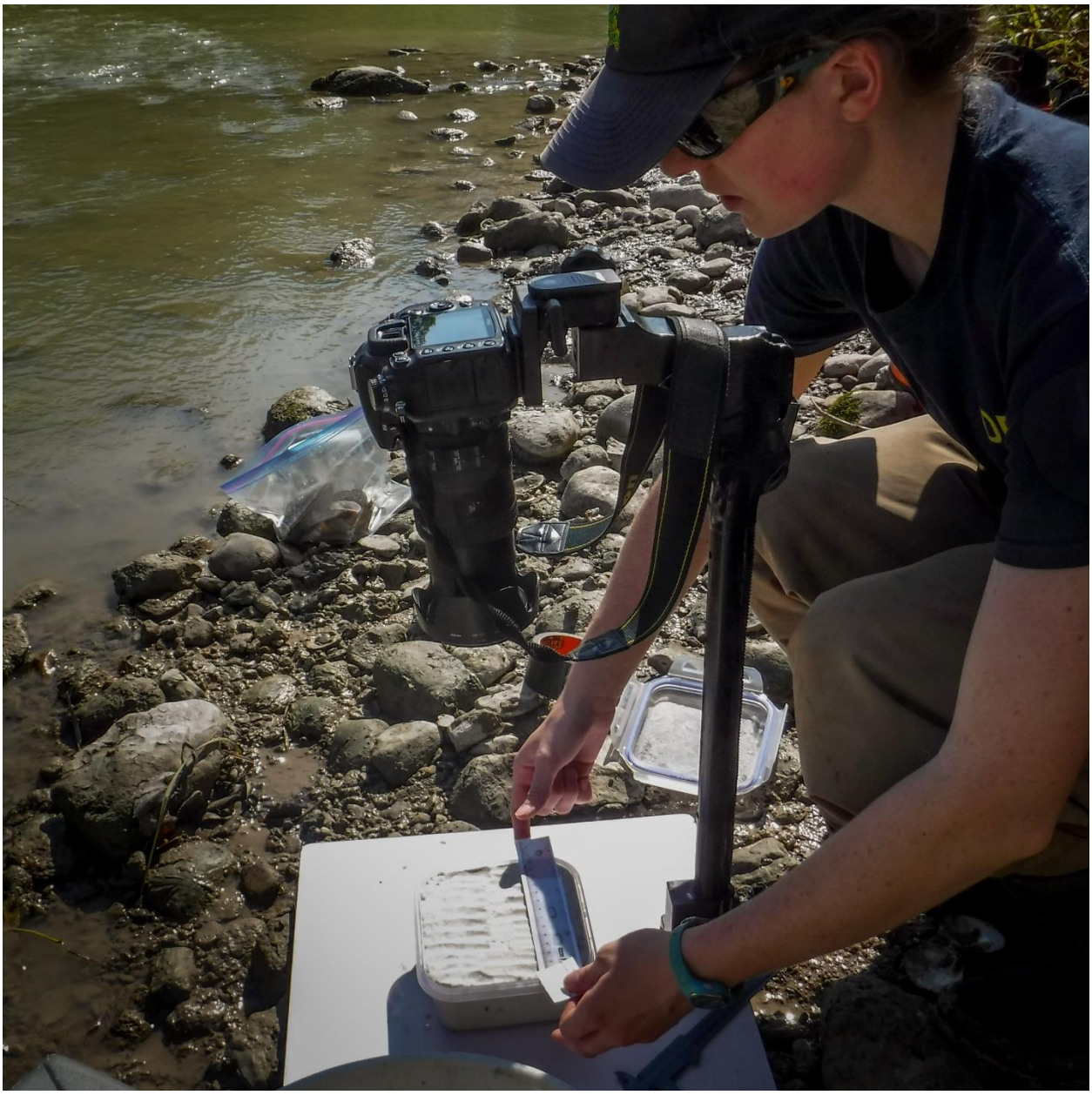

Figure 3. Photographic voucher setup demonstrating use of a copy stand. Note the sand container for positioning the specimen, presence of scale bar, and voucher label. Natural daylight allows for good colour rendition of the photographed specimens; reducing excess shading during sunny weather also yields better imaging. See Digital Voucher section for recommended details of voucher label.

Figure 4. Core voucher photos required for identification of most Canadian Unionida. Top: lateral view of left valve showing overall shape and beak position. Bottom: Dorsal view showing inflation of valves. Right: close-up view showing presence/absence and composition of beak sculpture. Photo credit Canadian Museum of Nature (top).

Figure 5. View of some of the collection cabinets on a compactor rail system containing Canada's primary freshwater mussel collection, at the Natural Heritage Building of the Canadian Museum of Nature (CMN), on 1740, Pink Road, Gatineau, Québec. Voucher specimens of freshwater mussels sent to the CMN are archived by professional collection staff. This collection can be consulted by malacologists, university students, consultants, and expert naturalists for taxonomical, ecological, biodiversity or conservation studies. Photo credit Canadian Museum of Nature.