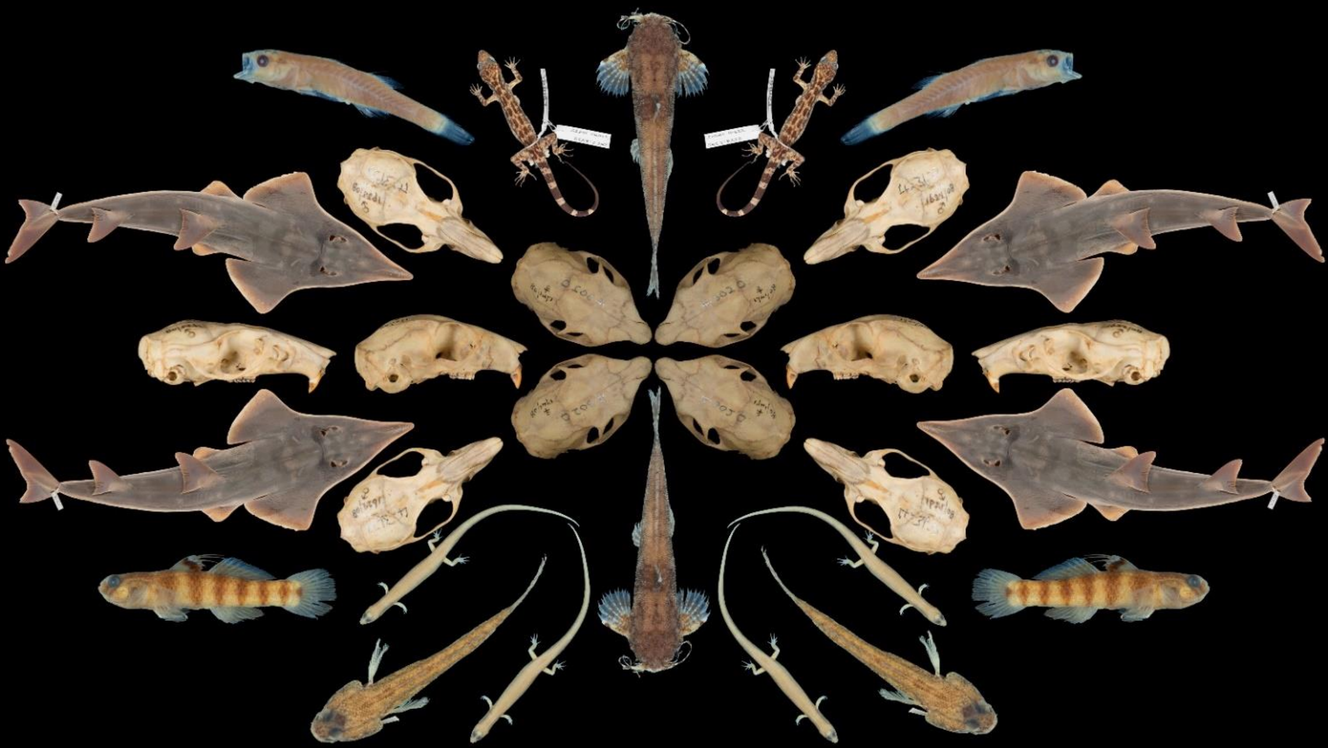
LEE KONG CHIAN NATURAL HISTORY MUSEUM

2022

VERTEBRATE TYPE SPECIMENS COLLECTED FROM SINGAPORE



A Digitisation Workflow

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Vertebrate Type Specimens Collected from Singapore: A Digitisation Workflow

By Christian J. W. Ching[1,2], R. Shivaram[2], Bi Wei Low[2], Heok Hui Tan[2], Marcus A. H. Chua[2], Kin Onn Chan[2], Kelvin K. P. Lim[2] & Wan F. A. Jusoh[2]

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Cover photograph: Type specimens of vertebrate species described from Singapore that are currently housed in the Zoological Reference Collection, Lee Kong Chian Natural History Museum. Species featured in this plate include *Parakysis longirostris*, *Gobiopterus birtwistlei*, *Mugilogobius fasciatus*, *Eugnathogobius illotus*, *Rhynchobatus cooki*, *Cyrtodactylus majulah*, *Tytthoscincus temasekensis*, *Callosciurus notatus miniatus* and *Maxomys surifer*. Cover design © Christian Ching; photographs © Christian Ching, Shivaram Rasu and Bi Wei Low.

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CONTENTS

LIST OF ABBREVIATIONS USED

BHL	Biodiversity Heritage Library
DNG	Digital Negative Image File
DSLR	Digital Single-Lens Reflex Camera
GBIF	Global Biodiversity Information Facility
iDigBio	Integrated Digitised Biocollections
ISO	Not an acronym as such, ISO is used as a metric to describe sensitivity to light in both film formats and digital camera sensors
LKCNHM	Lee Kong Chian Natural History Museum
NEF	Nikon Electronic Format (Nikon unprocessed, uncompressed image format)
NHMUK	Natural History Museum, London, United Kingdom
NUS	National University of Singapore
RAW	Unprocessed and uncompressed image format used by multiple camera systems, including Canon
RLM	Raffles Library and Museum
SEA	Southeast Asia
TIFF	Tag Image File Format
UID	Object Unique Identifier
ZRC	Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore

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INTRODUCTION

Of any major tropical region, Southeast Asia (SEA) has the highest relative rate of habitat loss, with current rates of deforestation projecting a loss of 13–85% of species by 2100 (Sodhi et al., 2010; Wilcove et al., 2013). A multitude of factors are driving regional extirpations and extinctions, including indiscriminate hunting, industrialisation and habitat loss through land-use change for agriculture or mining (Vijay et al., 2016; Hughes, 2017b; Gray et al., 2018; Zeng et al., 2018; Tilker et al., 2019). While the threats facing SEA habitats are becoming better understood, baseline data for many of the taxonomic groups of the region remain unavailable (Hughes, 2017a). Without understanding the larger ecological trends of SEA biodiversity, informing conservation actions, environmental policies and urban development becomes increasingly complex.

Natural history collections are the primary repositories of the information encompassing our planet's present and past biodiversity. These collections hold a trove of taxonomic, molecular, morphological and biogeographical data, and may also contain the only remaining representations of recently extinct species (Rocha et al., 2014). Even in the 21st century, research within collections has identified previously undocumented cryptic diversity within numerous SEA taxa, with wider implications towards the designation of protected areas (e.g., *Neofelis*, *Pongo* and *Trachypithecus*) (Buckley-Beason et al., 2006; Nater et al., 2017; Roos et al., 2020). With increasing rates of habitat loss and threats to their inhabitants, collection-based data is integral towards assessing the state of the natural world in addition to informing conservation actions (Meineke et al., 2018).

The Southeast Asian island of Singapore (103°50′E, 1°20′N), owing to its history as a British Crown Colony trading post between the South China Sea and the Indian Ocean, and the attention of early naturalists, has had its fauna and flora well documented and surveyed over the last two centuries (Chan & Davison, 2019). With its small size and well-established biodiversity baselines, the island has served as a natural laboratory for studies of tropical biodiversity loss across multiple taxonomic groups. The findings of such research have contributed towards the planning of conservation priorities and actions, through studies of population size and genetic diversity across taxa including birds, mammals, butterflies, plants and fish (Corlett, 1992; Castelletta et al., 2000; Wilcove et al., 2013; Kristensen et al., 2020; Theng et al., 2020).

The Lee Kong Chian Natural History Museum (LKCNHM) at the National University of Singapore (NUS) in 2020 began an initiative to digitally repatriate zoological specimens described from or in Singapore. This includes both specimens collected and purchased in Singapore. Involving natural history collections globally, the project aims to foster collaborative research between institutions and enhance critical research opportunities on SEA biodiversity. Data produced will be made available through open access repositories, enabling wider dissemination and collaborative generation of knowledge by institutions and researchers that may be unable to travel to physical collections outside of their country (Drew et al., 2017).

The LKCNHM virtual repatriation project aims to efficiently collect standardised data from many international natural history collections. In addition to publishing metadata, digitisation also serves the databasing of specimens, allowing for the updating of specimen catalogues within collections. Digitisation workflows are integral towards the production of standardised results. While previously published workflows for herbaria and entomological collections are available online, there has been little produced specifically for the digitisation of vertebrates (Nelson et al., 2015; Blagoderov et al., 2017). As part of the LKCNHM virtual repatriation project, the vertebrate type specimens of the Zoological Reference Collection (ZRC) of the LKCNHM were databased and digitised. Enhancing and increasing accessibility of digital type collections promotes the relevance and importance of these irreplaceable specimens and the institutions they exist within and ensures their preservation in the future.

This book presents a workflow for digitising type specimens, using vertebrate material collected from Singapore held within the ZRC as exemplar. Steps are outlined as they were performed within the LKCNHM, with suggestions given for alternative setups, including those used within other collections such as the Natural History Museum London (NHMUK). This workflow aims to be enhanced as the LKCNHM virtual repatriation project progresses, with future iterations including additional methodologies, lessons learnt and updated techniques.

MATERIAL AND METHODS

THE VERTEBRATE TYPE MATERIAL OF THE ZOOLOGICAL REFERENCE COLLECTION

Until the formation of the Raffles Library and Museum (RLM) in 1849 and the growth of its early natural history collections in the 1870s, the vast majority of zoological material collected in Singapore was sent to either London or Calcutta (Kolkata) (Tan, 2015). Much of the London material now resides in what is today the NHMUK. Even after the formation of the RLM, large numbers of type and non-type material continued to be sent to the NHMUK in order to provide greater access for European researchers and better specimen storage conditions (Tan, 2015). The material that remained in Singapore would eventually form the core collections of the ZRC under the care of the Raffles Museum of Biodiversity Research and currently the LKCNHM (Tan, 2015).

The ZRC boasts one of the largest repositories of Southeast Asian fauna within the region, having grown steadily over the decades since its formation. The collection holds over 834,000 specimens, and continues to grow with additions from expeditions, donations and other sources (Aw & Low, 2020). The holdings of the ZRC include 59 type specimens collected from Singapore representing 16 species: 31 mammals, one bird, 17 fish, four amphibians and 10 reptiles. These specimens were digitised from July to December 2020, during which methods and workflows were developed and refined. All specimen data captured were uploaded to the LKCNHM Biodiversity of Singapore portal (https://singapore.biodiversity.online) enabling virtual access to specimens.

WORKFLOW DESIGN

Our digitisation process has been adapted from the object-to-image-to-data workflow outlined by Integrated Digitised Biocollections (iDigBio) prepared for herbaria, with additional focuses within modules on the digitisation of vertebrate material (Nelson et al., 2012, 2015). The workflow (Fig. 1) has been developed with three primary modules comprising nine tasks. In the subsequent modules, each task is described further with explanations and notes made during the initial digitisation. The modules have been made to maximise the expertise of both digitisers and curators and to allow the digitiser independence earlier.

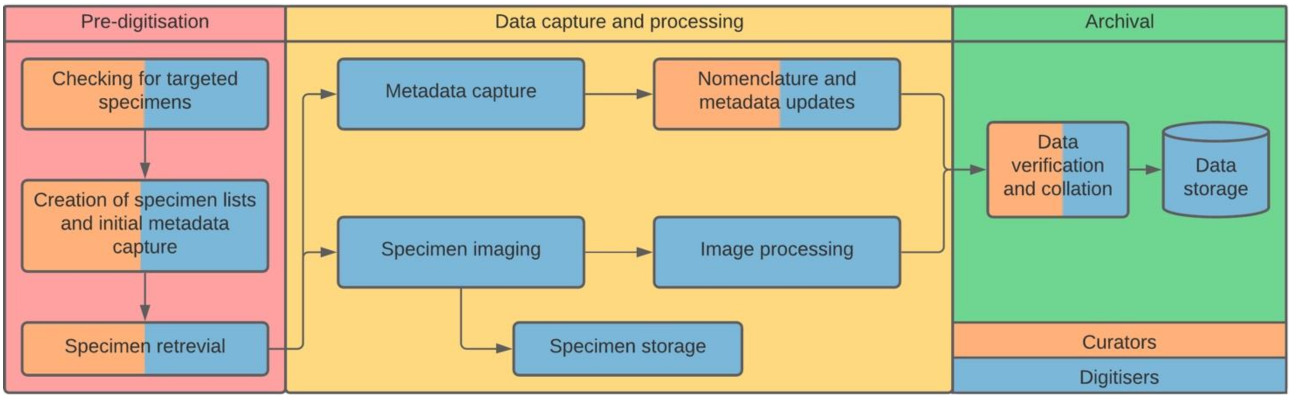

Fig. 1. Lee Kong Chian Natural History Museum Vertebrate Digitisation Workflow. Colours in action boxes indicate the staff responsible for each task.

IMAGING AND IMAGE PROCESSING

We captured specimen images using Nikon D800E digital single-lens reflex (DSLR) cameras (Nikon Corporation, Japan) mounted with Nikon AF-S Micro Nikkor 60mm f/2.8G or 105mm f/2.8 lenses, remote shutter releases and illuminated copy stands. One of us (CJWC) has had success using a Canon 6D MKII (Canon Inc., Japan) mounted with a Tamron SP 90mm F/2.8 Di Macro 1:1 272EE (Tamron Co., Ltd., Japan). Images were shot in the native raw format of the camera (. nef /. raw), to preserve the greatest amount of detail for downstream editing. It should be noted that while both cameras we used had full-frame sensors, high-quality images can be produced using crop sensor cameras and lenses. A smaller sensor size trades off some degree of dynamic range and resolution, but crop sensor cameras have shown to be effective in specimen digitisation, in addition to being generally cheaper and more portable (Brecko et al., 2014; Brecko & Mathys, 2020). We used Lightroom CC 2015.14 (Adobe Inc., USA) to process images for white balance correction and quality control. We processed images further in Photoshop CC 19.1.5 (Adobe Inc., USA) to remove backgrounds and add scale bars. We used Lightroom as it allowed for the mass processing and organisation of images while retaining file metadata, and Photoshop for the editing of individual files. In alternative methods of image-processing, software such as DxO OpticPro 11 (DxO Labs, USA) and Corel Photopaint (Corel, Canada) have been used for specimen digitisation and imaging (Häuser et al., 2005; Brecko & Mathys, 2020). We outline all steps taken to capture and process images. Adobe Lightroom and Photoshop are widely used today and are our preferred software, and we describe the processing workflows using these software in greater detail.

WORKFLOW MODULE 1: PRE-DIGITISATION CURATION

1.1 CHECKING FOR TARGETED SPECIMENS AND INITIAL METADATA CAPTURE

At the start, we generated specimen lists detailing the type material available within the ZRC. We received databases from curators, which served as initial sources of metadata. In cases where lists of targeted specimens are unavailable through curators, metadata can be captured through sources such as published literature (e.g., original descriptions, species checklists, bulletins), online repositories (e.g., museum databases, Global Biodiversity Information Facility [GBIF]), unpublished museum catalogues and transfer records. Each specimen list should be associated with a single taxonomic class, and the institution in question should be indicated. The list or file should follow the nomenclature, "CLASS_INSTITUTION", for example, "MAMMALIA_LKCNHM".

Once digitisers have been granted access to physical collections, they should also check for previously unknown or lost objects; these may be held together or associated with target specimens through unique identifiers (UID). It should be noted that many institutions including the LKCNHM may have several iterations of specimen UIDs used across different time periods, therefore curators should be consulted to understand these further.

1.2 SPECIMEN RETRIEVAL

We retrieved the ZRC type material and stored this in holding sites close to digitisation stations for ease of use. We inspected both labels as well as specimens for damage and photographed both unless the damage was excessive (i.e., if additional handling would cause irreparable damage). Specimens or labels in need of conservation were highlighted to curators. Any permanent damage to specimens was also noted, and we checked for pests such as fungal growth or invertebrates. If labels were damaged and unreadable, this was raised to curators to highlight conservation work and verify metadata (see Module 2).

Specimens **should not be repaired** by digitisers even if they have the necessary expertise unless given explicit permission by curators. Any damage caused during the handling of specimens must be reported.

WORKFLOW MODULE 2: SPECIMEN DATA CAPTURE AND PROCESSING

We conducted specimen data capture during specimen imaging and processing. Specimens and their associated specimen labels should always be imaged to facilitate data validation. Data should be substantiated by checking actual catalogue entries for additional information, as label sizes sometimes restrict the amount of data that can be written or printed. If time is limited, label data should be collected directly from specimen label images, allowing for enhanced readability through digital enlargement, and eliminating the need for several trips to storage locations (Nelson et al., 2012).

2.1 METADATA CAPTURE: SPECIMEN LABEL TRANSCRIPTION

Digital transcription of specimen labels enhances the usability of data for research and increases visibility within electronic database systems. Commonly captured data from labels may include the date of collection, locality, collector, scientific name (traditionally the original taxon name), identifiers and possibly several UIDs (see Fig. 2 for an example). While the LKCNHM and other major collections such as the NHMUK use a single UID for all parts of a single vertebrate specimen, other collections may have individual UIDs for different parts of a single specimen. In these cases, digitisers must ensure UIDs and objects are linked correctly.

We transcribed specimen data verbatim, by recording the exact text on the label, including errors. This minimised subjective interpretations of illegible writing. All labels were also photographed to allow for data verification and erroneous transcriptions to be updated in the future. To ensure the visibility and usability of data for subsequent studies, transcription focused on a number of core data components (Table 1), with data terms adapted from DarwinCore guidelines (Wieczorek et al., 2012). It should be noted that Table 1 includes georeferenced data. While we did not georeference specimens as all targeted collections were from Singapore, a guide to georeferencing can be found in Module 2.3 to benefit projects with larger specimen locality ranges. Sometimes specimen labels do not contain all this information, and effort should be made to find all associated metadata from material such as collector notebooks, correspondence or physical collection registers.

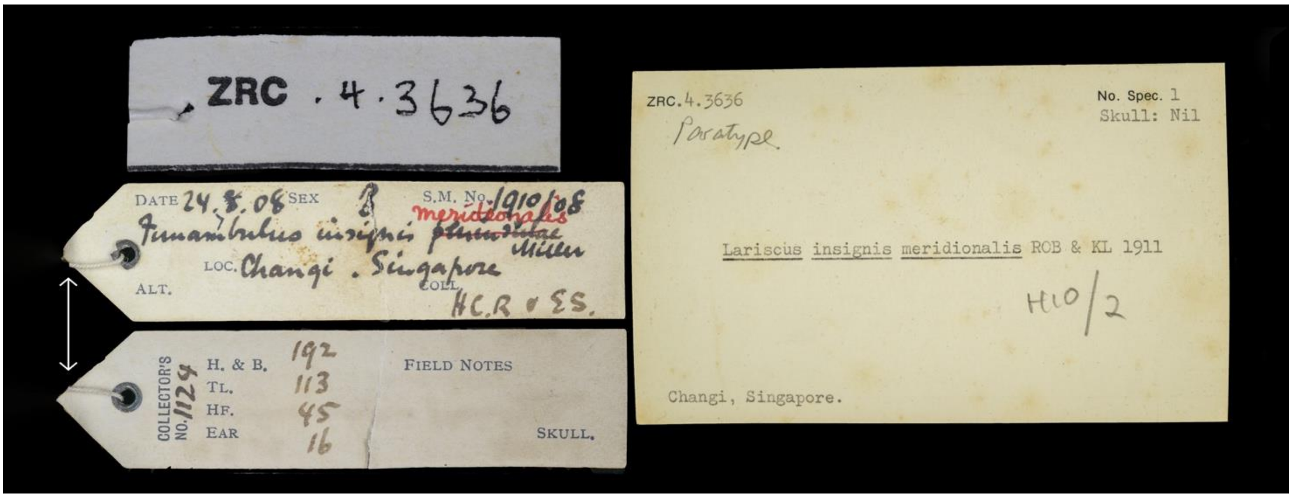

Fig. 2. Labels of *Lariscus insignis peninsulae.* The white double-headed arrow denotes two sides of a single label. Note that in addition to the unique identifier (UID) of the Zoological Reference Collection (ZRC), the label also indicates the UID of a previous museum (Selangor Museum 1910/08) and a collector number (1124). (Photographs by Christian Ching).

Table 1. Key metadata components transcribed and enhanced using collection labels to increase data usability. Definitions are adapted from DarwinCore data standards, an accepted stable reference for bioinformatic standards (Wieczorek et al., 2012).

Collections management	Definition	Example(s)
Object unique identifier (UID)	An identifier for the record within the data set or collection, including the institution code as a prefix.	ZRC 225A / NHMUK.47.1232
Other numbers/identifiers	An identifier given to the occurrence at the time it was recorded. Often serves as a link between field notes and institutional unique identifiers such as collector numbers.	CBK232 (Cecil Boden Kloss)
Class	The full scientific name of the class in which the taxon is classified.	Actinopteri
Order	The full scientific name of the order in which the taxon is classified.	Gobiiformes
Family	The full scientific name of the family in which the taxon is classified.	Gobiidae
Type nomenclature	The taxon name as it originally appeared when first established, followed by the name of the author(s) and date of description.	*Gobiella birtwistlei* (Herre, 1935)
Current nomenclature	The full name of the currently valid or accepted zoological taxon, followed by the name of the author(s) and date of description.	*Gobiopterus birtwistlei* (Herre, 1935)
Type status	The nomenclatural type status applied to the subject.	Holotype, paratype
Preparations	A list of preparations and preservation methods for a specimen.	Skin, skull, skeleton, whole animal, tissue
Specimen location	The current location of the specimen's preparations.	NHMUK South Kensington North West Tower 4/11/1
Locality	The original textual description of the collection location.	Changi
Country	The name of the country or major administrative unit in which the locality occurs.	Singapore
Collection date	The date-time or interval in which the collection event occurred. Records should be in DD/MM/YYYY.	24/08/1908
Sex	The sex of the biological individual represented in the occurrence.	Male / female
Original description reference	The reference for the publication in which the type's taxonomic determination was originally established.	Robinson, H.C. & Kloss, C.B. 1911 Journal Fed. Malay St. Mus. 4, p241
Georeferenced metadata	Georeferenced coordinates, extent and certainty scores. Details on data fields are found in Module 2.3.	
Other information on labels	Any other information on the label(s), recorded as verbatim.	Caught in a trap under coconut tree

2.2 TAXONOMIC VERIFICATION

Valid taxonomy is integral for effective research, and as science has advanced, so have accepted species concepts and methods of delineating species (Zachos et al., 2013). Over the course of time, it should be expected that the taxonomic information of type specimens may change with the description or synonymisation of species. Accurately updating specimen taxonomy is largely a technical exercise, and digitisers are not expected to be experts within their respective assigned taxa.

We collected specimen taxonomic information from labels verbatim, filling fields for both original nomenclature and any updated names, if present. Following the capture of all available metadata components, digitisers should consult curators to obtain currently valid/accepted nomenclature.

Institutions generally use specific publications to update the valid name of each taxon in their collection, allowing for standardisation. For example, the NHMUK uses Wilson and Reeder's Mammals of the World (Wilson & Reeder, 2005), the American Museum of Natural History Amphibian Species of the World Online Reference (Frost, 1999) and Eschmeyer's Catalog of Fishes (Frike et al., 2021).

Within some institutional instances, species names or higher classifications may follow older (outdated) terminology. In such cases, it may be worth inserting additional data columns for institution-specific taxonomic names (e.g., "institutional genus" and "institutional species") if these are not currently accepted, in addition to the basionym (original name and original combination) and currently accepted species name.

2.3 GEOREFERENCING

Locality information recorded on specimen labels, particularly on historic specimens, is frequently vague, cryptic or faded with time. Georeferencing is necessary to ensure specimens are associated with the correct region, country or island. Our digitisation effort did not include georeferencing as we only used specimens collected from Singapore, an island of approximately 50 km at its greatest length. Nonetheless, georeferencing enhances specimen data and should be included in digitisation projects with localities spanning larger geographies. The short process guide below is based on established practices from Wieczorek et al. (2004) and Chapman & Wieczorek (2020).

Georeferencing should use the GBIF Best Practice Guidelines (Chapman & Wieczorek, 2020). Specimen localities should be updated if needed, using geographical databases such as GeoNames (http://www.geonames.org/) or other gazetteers. Resources available online such as publications mentioning localities may also be useful for ascertaining updated names of places. Locations should then be found on Google Maps, with decimal degree latitudes and longitude coordinates for the geographic centre of the locality recorded. Procedures for determining geographic centres are dependent on the type of locality; most named places such as villages, mountains and provinces have clear midpoints. Other locality types such as rivers should be measured by drawing a straight line from the mouth to the head of the river, and caves georeferenced at their entrances. Detailed guidelines for each type of location can be found in Wieczorek et al. (2004) and Chapman & Wieczorek (2020).

Using the point-radius method, adapted from MaNIS / HerpNEt / ORNIS guidelines (Wieczorek et al., 2004), measures of error should also be included through extents and certainty scores. Extents should also be recorded from midpoints as radiuses describing the maximum distance from the midpoint in which the locality is expected to occur, and certainty scores (0, 25, 50, 75, 100%) describing the confidence that the described locality falls within the recorded extent.

Google Maps allows for efficient georeferencing; right clicking on a geographic point shows its coordinates, and the measure distance option can be used for measuring extent values. Ensure that coordinates are entered the right way around, and attention is paid to possible negative values. Throughout the georeferencing process, it is important to make extensive notes describing why you chose the coordinates and extent, allowing anyone else examining the locality information to follow your reasoning and get the same locality values. In addition, it should be noted that some localities just cannot be georeferenced, and that no data is better than incorrect data!

2.4 SPECIMEN IMAGING

Imaging of specimens and labels can benefit curation by facilitating the aggregation of high-quality information (e.g., metadata, morphology) from specimens that may not have been previously captured, while minimising future physical handling of historical and/or fragile objects, thereby reducing the risk of damage (Kalms, 2012; Van Walsum et al., 2019). We did not remove labels from specimens prior to photography but in certain cases, doing so may be beneficial towards revealing diagnostic characteristics. If labels need to be removed, it is imperative to check with and follow the guidance of curators prior to altering any part of a specimen.

Our aims were to produce images of specimens and labels that could be used for both exhibitions and outreach, as well as research purposes such as taxonomic verification and morphological identification. Multiple interpretations of 'high-quality' images exist. Publications on the two-dimensional imaging for snakes and fish specimens regard 'high quality' as images that accurately represent the specimen in addition to being sharp, of high resolution and properly exposed (Herler et al., 2007; Tan, 2014; Kaiser et al., 2018). In-depth working knowledge is needed to photograph diagnostic characteristics of each taxonomic group, so this approach would not be viable for most digitisers on multi-taxa projects. When digitising types, we found it beneficial to refer to the specimen's original description to ascertain any characteristics that may have been used to initially describe the species.

For our digitisation project, we photographed whole specimens in dorsal, ventral and lateral views (if applicable), and both sides of all tags and labels. This approach allows for further use of images, including digital morphometric and meristic studies (Herler et al., 2007; Vu et al., 2018; Cardini et al., 2021) (Figs. 3–5). We photographed all specimens facing left, with left sides captured in lateral perspective to standardise digitisation outputs. This follows the imaging conventions of fish (Tan, 2014), and encourages the viewing of specimens from anterior to posterior in line with a left-to-right reading direction. In some cases, both sides of specimens may be photographed to capture abnormalities or asymmetry. If left sides were more heavily damaged than right sides, specimens were flipped and their right sides photographed. Such images can be flipped during digitisation to follow conventions in image presentation, but this flipping must be clearly indicated (i.e., that images were taken from the right) as certain taxonomic groups, including mammals, amphibians, fish and reptiles, may exhibit bilateral symmetry (Brown et al., 2017; Costa et al., 2017; Raffini & Meyer, 2019; Coombs et al., 2020). For warped labels, we wedged them between the background and a glass pane before photography. It should be noted that when photographing labels, it is best practice to ensure that all labels are aligned with the horizontal guides of the viewfinder. This will save time when processing images at later stages.

Fig. 3. Dorsal aspect of *Microhyla mantheyi*, showcasing a suite of commonly used morphometric measurements that can be obtained from an imaged frog specimen. Each measurement is represented by a number on the figure. 1, eye-nostril distance; 2, internarial distance; 3, interorbital distance; 4, head width; 5, forearm length; 6, snout-vent length; 7, finger disk width; 8, thigh length; 9, foot length; 10, tibia length. (Photograph by Shivaram Rasu & Low Bi Wei).

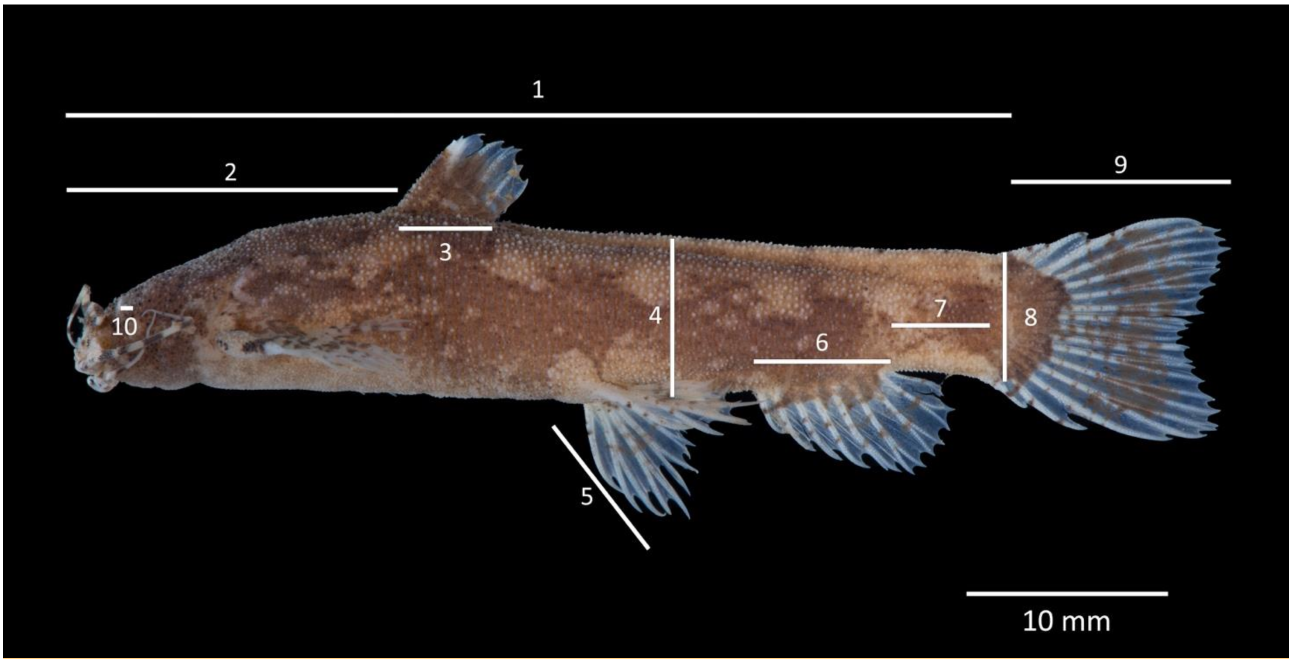

Fig. 4. Lateral aspect of *Parakysis longirostris*, showcasing a suite of commonly used morphometric measurements that can be obtained from an imaged fish specimen. Each measurement is represented by a number on the figure. 1, standard length; 2, predorsal distance; 3, dorsal fin length; 4, body depth at anus; 5, pelvic fin length; 6, anal fin length; 7, postanal peduncle length; 8, caudal peduncle depth; 9, caudal fin length; 10, eye diameter. (Photograph by Shivaram Rasu & Low Bi Wei).

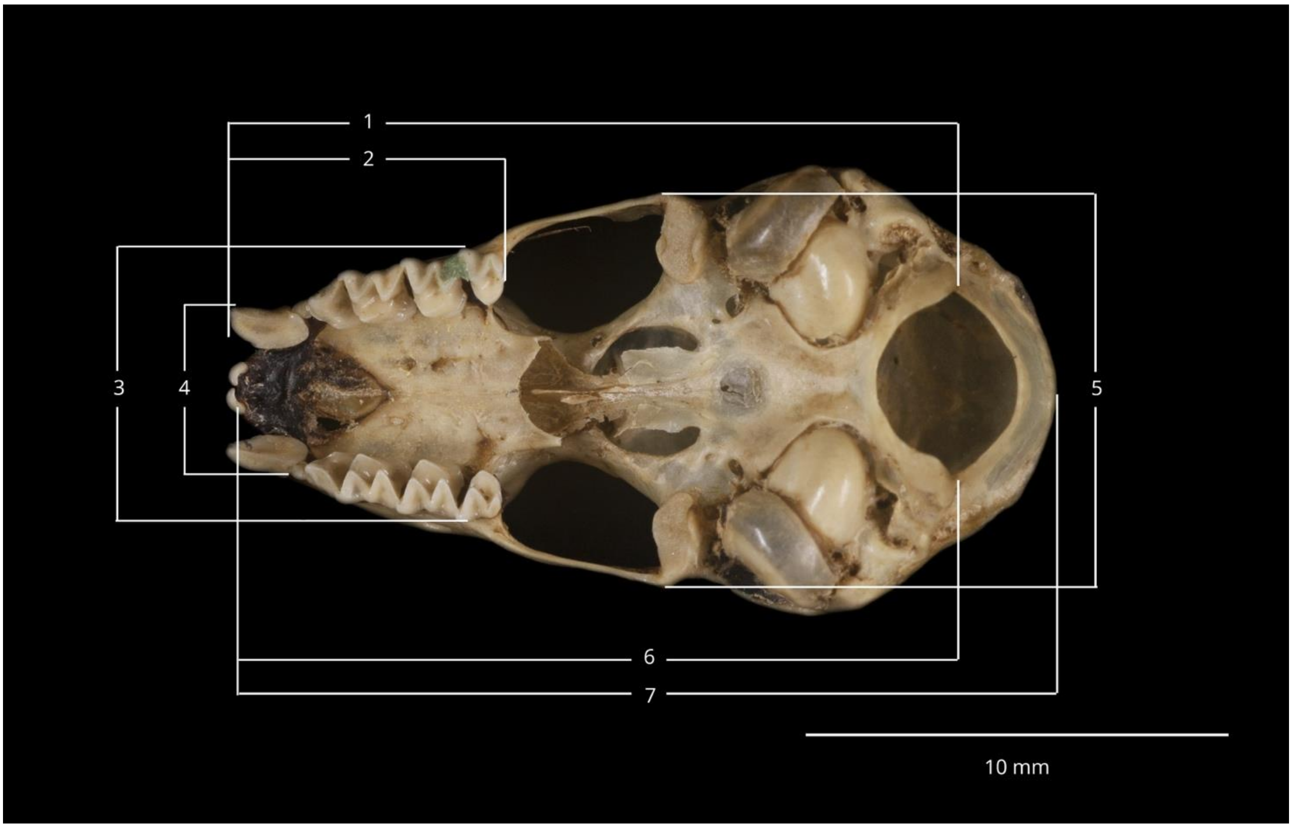

Fig. 5. Ventral aspect of *Hipposideros ridleyi*, showcasing a suite of commonly used morphometric measurements that can be obtained from an imaged mammal specimen. Each measurement is represented by a number on the figure. 1, condylo-canine length; 2, maxillary toothrow; 3, posterior palatal width; 4, anterior palatal width; 5, zygomatic breadth; 6, condylo-basal length; 7, grand total length. (Photograph by Christian Ching).

All images should include a scale (Fig. 6c). Suitable examples include placing a conventional ruler or a forensic ruler (which may work well, as many iterations contain colour bars) beside the specimen. In cases where a physical scale cannot be placed alongside a specimen (e.g., specimens suspended in liquid medium or air), the specimen can be physically measured separately, and a scale bar added subsequently during image processing. To minimise other distortions and aid future comparisons, specimens should be photographed on a level plane using a bubble leveller for reference. Guidance on positioning is included in later modules. Throughout the imaging stage, digitisers should regularly check images for quality and sharpness.

Fig. 6. Raw photos of *Mus surifer leonis.* Note that specimens are facing left (a), have been placed away from the edges of the image (b), and include a scale bar (c). (Photographs by Christian Ching).

2.4.1 LIGHTING

While image exposure can be optimised with sufficient knowledge of the camera's shooting settings, appropriate lighting setups will drastically increase output quality. Specimen photographs should be evenly lit, with no glare or reflections. Our imaging stations were in rooms with darkened or no windows and lit with artificial light to prevent variable light conditions that may produce unwanted shadows and reflections. In the LKCNHM, we used copy stands with constant lights (Kaiser Fototechnik, Germany) that were set up for static photographic documentation processes. Lighting setups may vary across institutions, and if constant lighting is not available, strobes or camera flashes can be utilised, but keep in mind that these sharp bursts of light will create harsh shadows. For Nikon users, a portable and very efficient setup to use is the R1C1 macro light kit. This kit is wireless, using IR for strobe triggering. Many small strobes (SB-R200) can be used in conjunction with larger flash units (SB900 and higher models), using the SU-800 commander attached to the DSLR camera hotshoe to provide adequate lighting.

Fig. 7. A copy stand with sheets of tracing paper wrapped around the lights, acting as diffusers to soften luminosity. High degrees of reflectivity off the shown mousedeer pelage were visible in photographs until the diffusers were added. (Photograph by Christian Ching).

We made diffusers from tracing paper (Fig. 7) to soften light and reduce reflections and harsh shadows on the specimens. This is especially helpful for photographing specimens with iridescent or glossy exteriors. If dedicated light diffusers are unavailable, alternatives can be made using thin paper or fabrics. Light should also be directed onto the specimen surface at an angle to reduce glare reflected into the lens.

2.4.2 CAMERA SETTINGS

Digitisation typically uses professional standard cameras to image specimens. Currently, the two primary types of cameras are digital single-lens-reflex (DSLR) and the newer complementary-metal-oxide-semiconductor (CMOS) sensor mirrorless cameras. As DSLR cameras have been in the market longer, they have a wider range of lenses and accessories available, usually for lower prices. When imaging specimens, macro lenses are generally used to ensure subject perspective along with photograph sharpness and quality, as they are able to focus on objects within close range of the lens. (1:1 life size ratio).

Table 2. Specimen photograph camera setting details.

Basic setting	Details
Imaging station camera setup	A tripod or copy stand should be used to ensure stability and overall consistency of images produced. It also allows for longer shutter speeds, which will allow for higher f-stops and greater depth of focus. In addition, a remote shutter release or computer tether could be used for the shutter release, to minimise camera movement during image capture. If a cable release is unobtainable, the camera should be set on timer or exposure delay mode to allow for residual camera movement to subside before capturing the image. For advanced DSLR camera models, a mirror-up function should be actuated to reduce minute vibrations due to mirror flip. For mirrorless camera setups, this is not necessary.
ISO	Generally, ISO values should always be at their lowest to minimise noise and maximise dynamic range in the image. However, lowest ISO settings can sometimes be expanded settings that pull exposure from software and may decrease image dynamic range. Photographs taken with an ISO of between 100–200 is ideal.
Aperture / f-Stop	The maximum depth of field should be captured without compromising imaging quality. It is recommended to use an f-stop of 16–22 to maximise sharpness and depth of field. As most labels are flat, an aperture of f/8 can be used for capture of label data.
Camera mode and metering	The digitiser may decide to photograph in the aperture priority or full manual shooting modes of the camera. Aperture priority designates that the shutter speed will be controlled by the camera. If using aperture priority, spot metering should be used to set the proper exposure of the subject (the choice of spot metering should be adjusted accordingly to each subject). For subjects with contrasting tonal patterns, it may be difficult to standardise exposure across images of different aspects (e.g., dorsal, ventral, lateral). In these cases, exposure can be controlled by metering up and down, or by shooting in full manual mode, using consistent apertures and varying shutter speeds.
Shutter speed (if using full manual)	When capturing images with full manual control, aperture and ISO values should be kept within the limits described above, and shutter speed used to control the overall exposure of the image. If the camera is stabilised by a copy stand or tripod, longer shutter speeds may be used, as long as both the camera and specimen remain stationary.
File format	The camera should be set to save photos in raw image formats, which are uncompressed digital negatives. This would be RAW files for Canon users and NEF files for Nikon users. Digital negative files hold more data than traditional image formats such as JPEG, and increase dynamic range while allowing for more options during post processing without loss of quality.
Focusing	Resulting images should aim to keep most of the specimen in focus, allowing for their use in morphometrics and meristics. Accordingly, photographers should keep aperture-based depth of field in mind, with focus points of images being either diagnostic characteristics (e.g., dentition in ventral mammal skull images) or medial areas (to allow for greater focal coverage) of the specimen. In most cases, autofocus settings are appropriate for imaging. However, in some cases, such as when imaging reflective specimens, the autofocus mechanism may have trouble finding a focal point. With these specimens, manual focusing should be used. When focusing on a specimen, it is useful to switch to the 'live view' function of the camera, if available, and increase the magnification on the monitor to ensure targeted points are in focus. During the photography of labels, it is best to sandwich all labels under a pane of glass, and use the text on a single label for focus.

The macro lenses we used included a Tamron SP 90mm F/2.8 Di Macro 1:1 272EE as well as a 60mm f/2.8G and a 105mm f/2.8 Nikon AF-S Micro Nikkor. Macro lenses with focal lengths of approximately 50 mm work efficiently as good all-rounders, while lenses with shorter focal lengths may be used to image larger specimens, but may suffer from wide angle distortion (which can be fixed in post processing; see Module 2.4.2). Conversely, lenses with longer focal lengths may be used for smaller specimens.

Throughout the imaging process, we reviewed the depth of field, focus and quality of photographs after each shot to minimise the need to re-photograph specimens later. To optimise imaging exposure, focus and overall quality, the digitiser should understand the setup of photographic stations, operation of the camera and shooting settings (Table 2).

2.4.3 DRY SPECIMENS

Photography of dry specimens such as mounted taxidermy specimens, flat skins and skulls is largely straightforward, but care should be taken when handling specimens to avoid damage. Issues may arise when trying to position specimens during photography to ensure they remain still and on a flat plane relative to the camera. We resolved this by using weighted beads underneath a velvet cloth background on which the subject rests (Fig. 8). Other methods such as the use of plastic bracelet beads, modelling putty or beanbags have been used in the past with effective results. It is important to consult curators regarding the methods permitted for their specimens; some do not allow the use of modelling putty as it can damage or leave residue on the specimens.

Fig. 8. Use of lead beads to position a mandible laterally, without and with a velvet cloth (cut with a hole to allow a single side of the mandible to pass through). (Photographs by Christian Ching).

2.4.4 WET SPECIMENS

Most, if not all, fluid-preserved specimens are stored within cylindrical containers, which increases reflection and distortion during photography. As such, imaging of wet specimens should take place in specialised glass tank setups (i.e., wet boxes or squeeze tanks). These containers allow for the positioning of the specimen within a fluid, which results in more "natural" looking photos; this is owing to certain morphological features (e.g., fins of fishes, barbels of fishes, setae in soft-bodied organisms) collapsing or folding up when exposed to the air. Deionised or distilled water should be used to minimise bubbles forming on the glass or the specimen, although if tap water is to be used, it should be left to allow impurities to settle before initiation of photography.

Specimens that are stored in 75% ethanol should be hydrated pre-photography. This is achieved by placing the subject in water for 15 to 30 minutes (size dependent) prior to photography. This hydration process will alleviate image distortion due to mixing of ethanol and water, and unwanted liquid gradients. Specimens can then be gently mounted on a propping pin to allow for the photography of different aspects of the individual, while minimising handling time of the specimens (see Fig. 9). The propping pin is embedded in a block of clear acrylic and helps to suspend the specimen in a liquid medium. Again, it is integral that curators are consulted beforehand on permitted methods, as the use of a propping pin will cause damage to the specimen. Other non-destructive methods such as squeeze tanks or the laying of specimens directly on the bottoms of containers may also be used.

If multiple specimens are shot in a single session, the tank may have to be drained and refilled at regular intervals to minimise the build-up of particulates and visible impurities in the liquid that may show up in photographs.

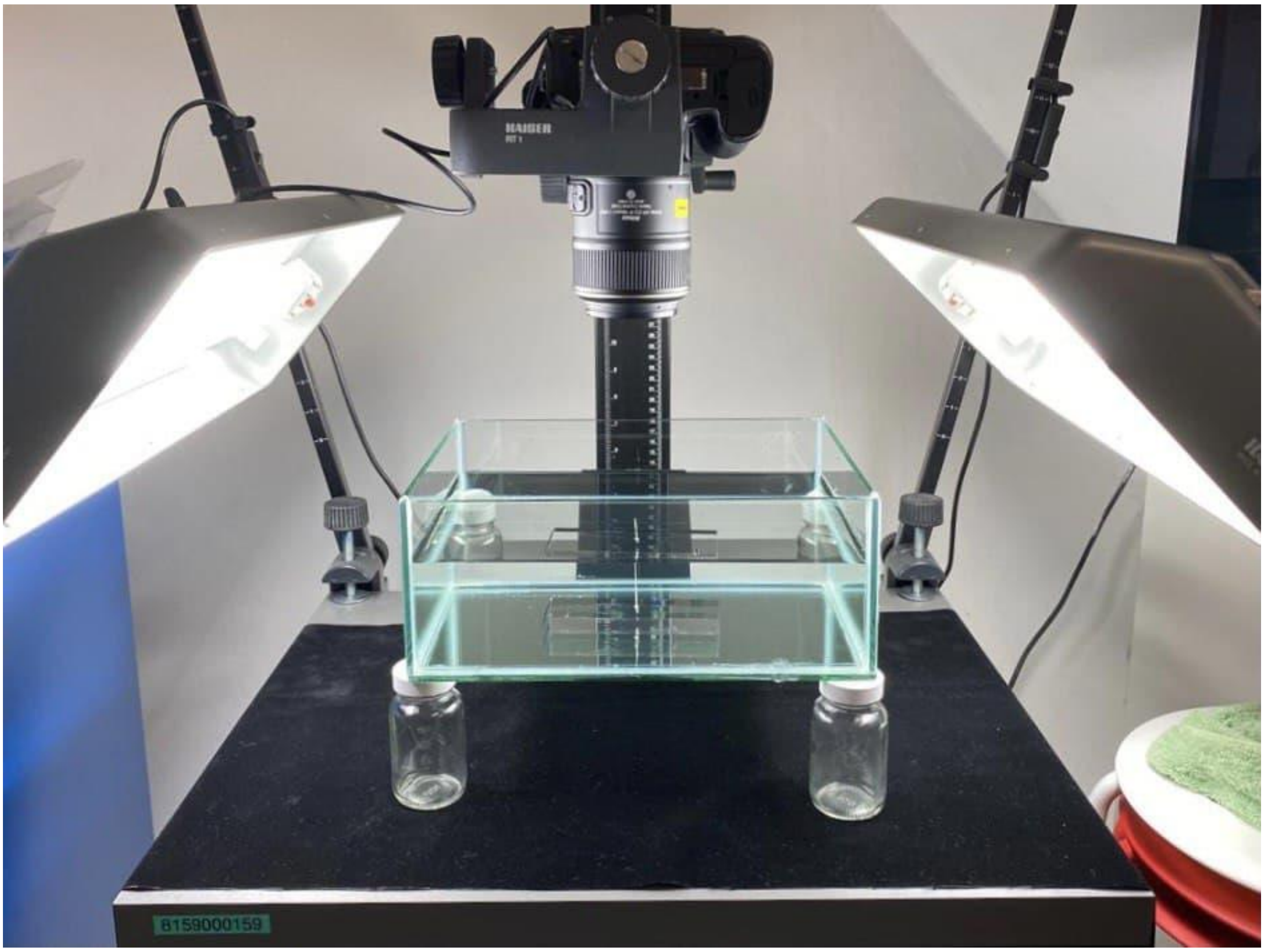

Fig. 9. Tank filled with water with propping pin for imaging wet specimens, using a copy stand with lights. The glass tank is elevated to provide distance blurring of background, and also to facilitate change of background colour (if so desired, e.g., to reveal black markings on translucent fins of fish). (Photograph by Shivaram Rasu).

For larger specimens, use of a larger glass tank setup is recommended. If a specimen is too large to be photographed within a container, it should be patted dry to minimise reflections on the surface of the specimen, photographed like a dry specimen, then returned to its storage container as quickly as possible.

2.5 IMAGE PROCESSING

Our final image output consisted of a figure plate image file containing all photographed aspects of a single specimen and any associated data labels. We first processed digital negative files from the camera cards in Adobe Lightroom for initial quality control. This included deletion of repeated images as well as an additional stage of checking for blurred or poor images. Following this, we colour-balanced and saved files in Adobe Digital Negative (DNG) format, a file type that is easily accessed by multiple image-editing programmes. We then processed DNG files into figure plate images in Adobe Photoshop, with files saved in the Tag Image File Format (TIFF), which is lossless. Digitisers should familiarise themselves with the basic functions of both applications. Detailed methods of image processing are outlined below.

2.5.1 FOLDER PATHS AND NAMING CONVENTIONS

Post-imaging, we stored image files on an external hard drive and renamed them to identify the object, using the following naming convention of:

(INSTITUTION_ID.SPECIMEN_NUMBER.SPECIES.OBJECT.ASPECT). For example, a dorsal image of the skull of *Lariscus insignis meridionalis* (Robinson & Kloss, 1911) would be:

(ZRC.4.3636.Lariscus.insignis.meridionalis.skull.dorsal).

Organisation of collected data is vital for the efficiency of further file processing. We ensured folder paths were consistent (Fig. 10), with standardised file saving sites to facilitate data access by any project member. Each specimen had a single folder, with subfolders for camera digital negatives, DNG or processed files. Metadata sheets were also saved within the folder denoting taxonomic class.

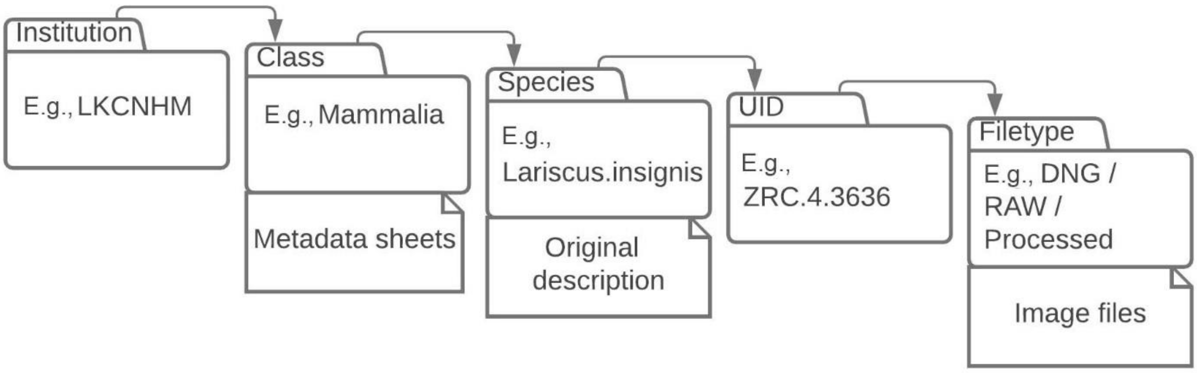

Fig. 10. File pathing layout.

2.5.2 LIGHTROOM FILE PROCESSING

Once we completed renaming, organisation and saving of all image files and metadata sheets, the institutional root folder (including its subfolders) was imported into Adobe Lightroom (Fig. 11a). This was done to select the best images, if multiples had been captured, and to colour-correct the image. The sharpest and clearest images were selected, with repeated instances deleted.

Colour was corrected through first navigating to the 'Develop' tab (Fig. 11b) of Lightroom and selecting the eyedropper tool (Fig. 12a). Lightroom adjusts the temperature and tint of images through the selection of a target neutral, which is an area with similar red, green and blue values. We used white or grey cards as target neutrals, but dedicated colour charts are often used in wider specimen photography efforts.

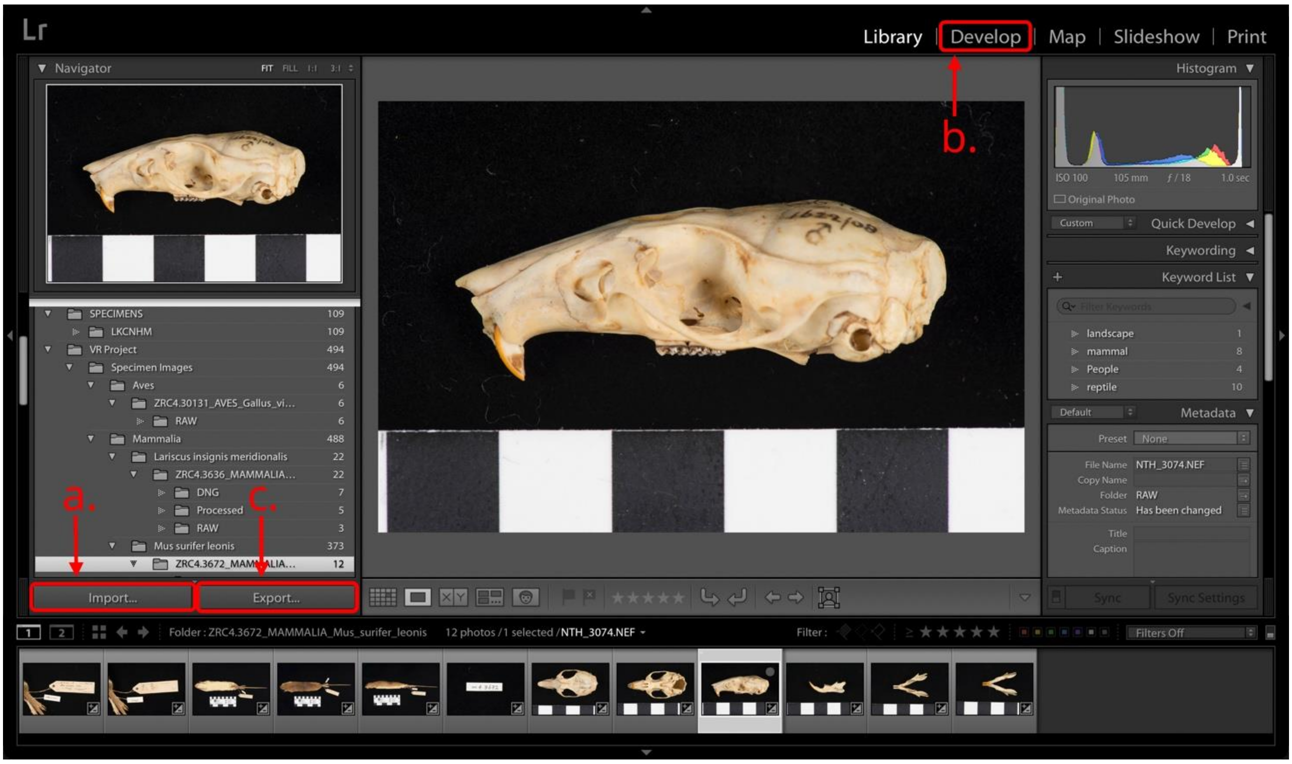

Fig. 11. Adobe Lightroom 'Library' tab showing import dialog box (a), 'Develop' tab (b) and export dialog box (c).

Once the colour was corrected in a single image, we used its corrected white balance values to correct the remaining images taken during the same photographic session. This could be done as we photographed specimens in artificial light that did not change over the course of each session. To do this, the colour balance of the image was copied by selecting 'Copy settings' at the bottom of the left Lightroom pane, then replicated across all images taken during the same session by selecting multiple images and using the 'Paste settings' function (Fig. 12b).

Following processing in Lightroom, images were exported back into their individual specimen digital negative subfolders as DNG files. This was done through selecting "DNG" as the image format within the file settings tab in the export window (Fig. 11c).

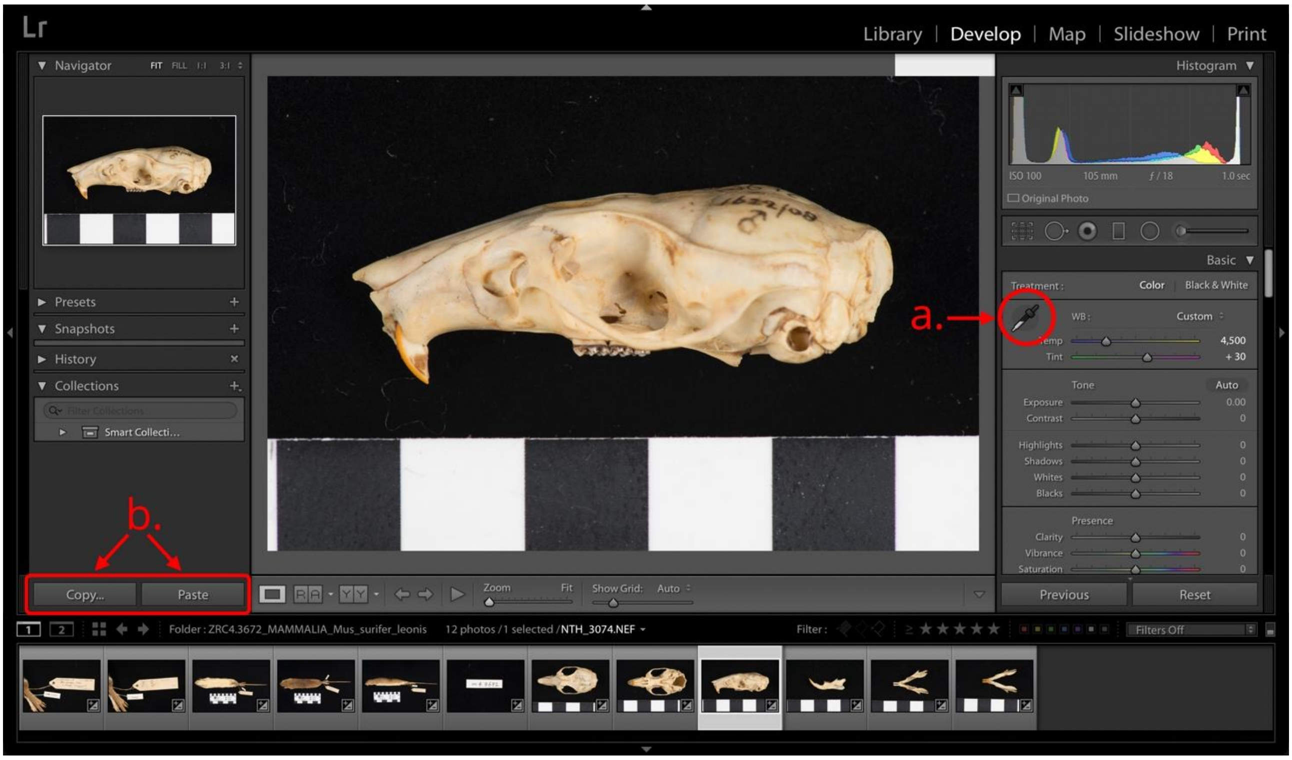

Fig. 12. Adobe Lightroom 'Develop' tab showing colour balance eyedropper (a) and develop setting functions (b).

2.5.3 PHOTOSHOP FILE PROCESSING

Once exported, we used Photoshop to add scale bars and remove background elements. We first created scale bars using the 'line' tool against the measurement scale used during imaging. Appropriate increments of length should be used, displaying a fraction of the specimen's extent. The text denoting length displayed was placed under the scale bar and set in the Open Sans font (see figure plates in the Appendix). Labels do not need a scale bar; however, a double-headed arrow should be used to indicate two sides of a single label (Fig. 13).

To remove background elements such as fur, feathers, dust, glare, bubbles or any other distortions, we first selected the entire specimen. We did this using a combination of tools such as 'magic wand', 'quick-select' and 'magnetic lasso', depending on the specimen or the label. We found that the 'select subject' function in Photoshop is particularly useful for objects that have clear outlines such as herpetological specimens, mammal skulls and labels. However, the 'select subject' function has difficulties selecting specimens with rough outlines and thin anatomical structures such as fur and whiskers. When using the 'select subject' function, we ensured that no characteristics were deleted and that the entirety of the specimen was selected. If areas were found to be unselected, we added them to the selection area manually by holding the 'shift' key down while selecting targeted areas. Alternatively, if backgrounds are composed of a solid colour, the 'colour range' tool from the 'Selection' menu in Photoshop can be used to select entire backgrounds, with fuzziness levels adjusted to ensure only the background is selected. Once specimens were satisfactorily selected, the feather tool was used to soften the selection edges, followed by the 'invert selection' function to select all background elements which could then be deleted.

In cases where the shades of a black background were inconsistent (velvet backgrounds generally never show as true black on images), a black fill layer was created on top of the specimen and the 'Underlying Layer' setting adjusted. The adjustment arrow was split into two, using the 'option' key (OSX) or the 'alt' key (Windows) (Fig. 14).

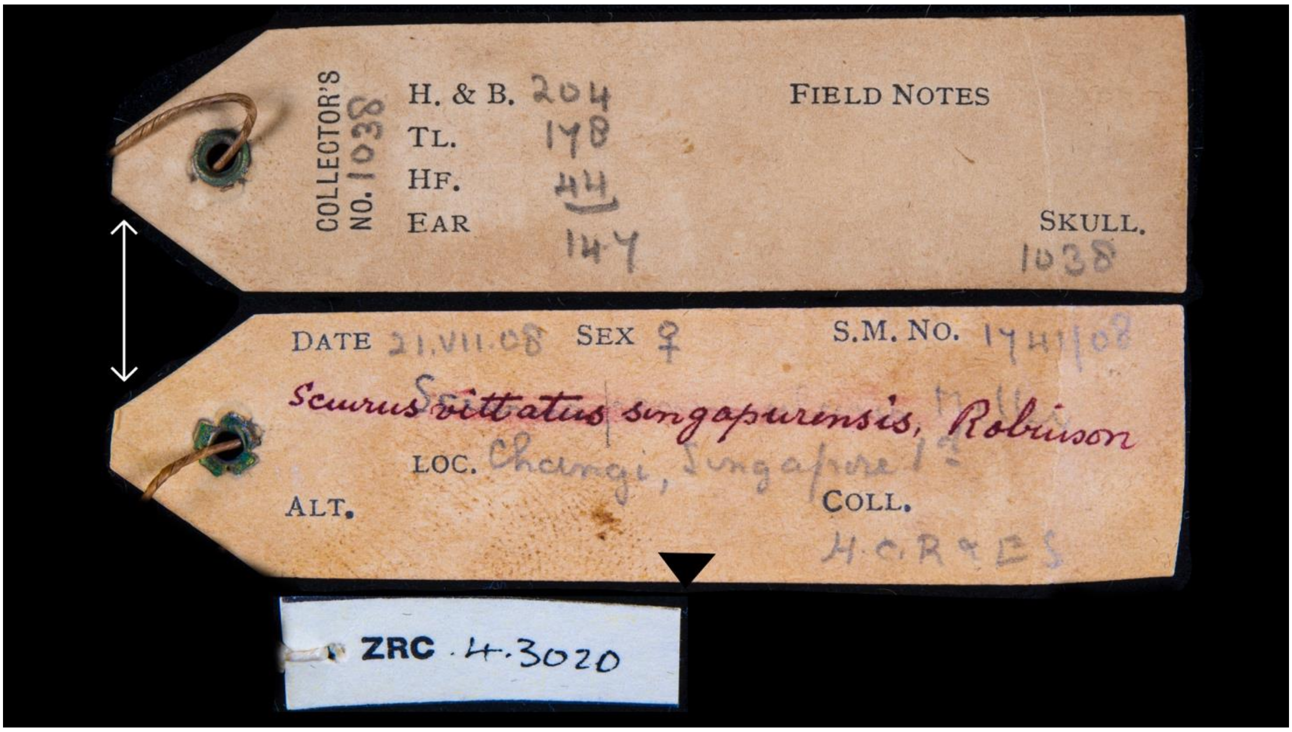

Fig. 13. Labels of *Sciurus vittatus singapurensis*, ZRC.4.3020. Note the double-headed arrow indicating a double-sided label. (Photographs by Christian Ching).

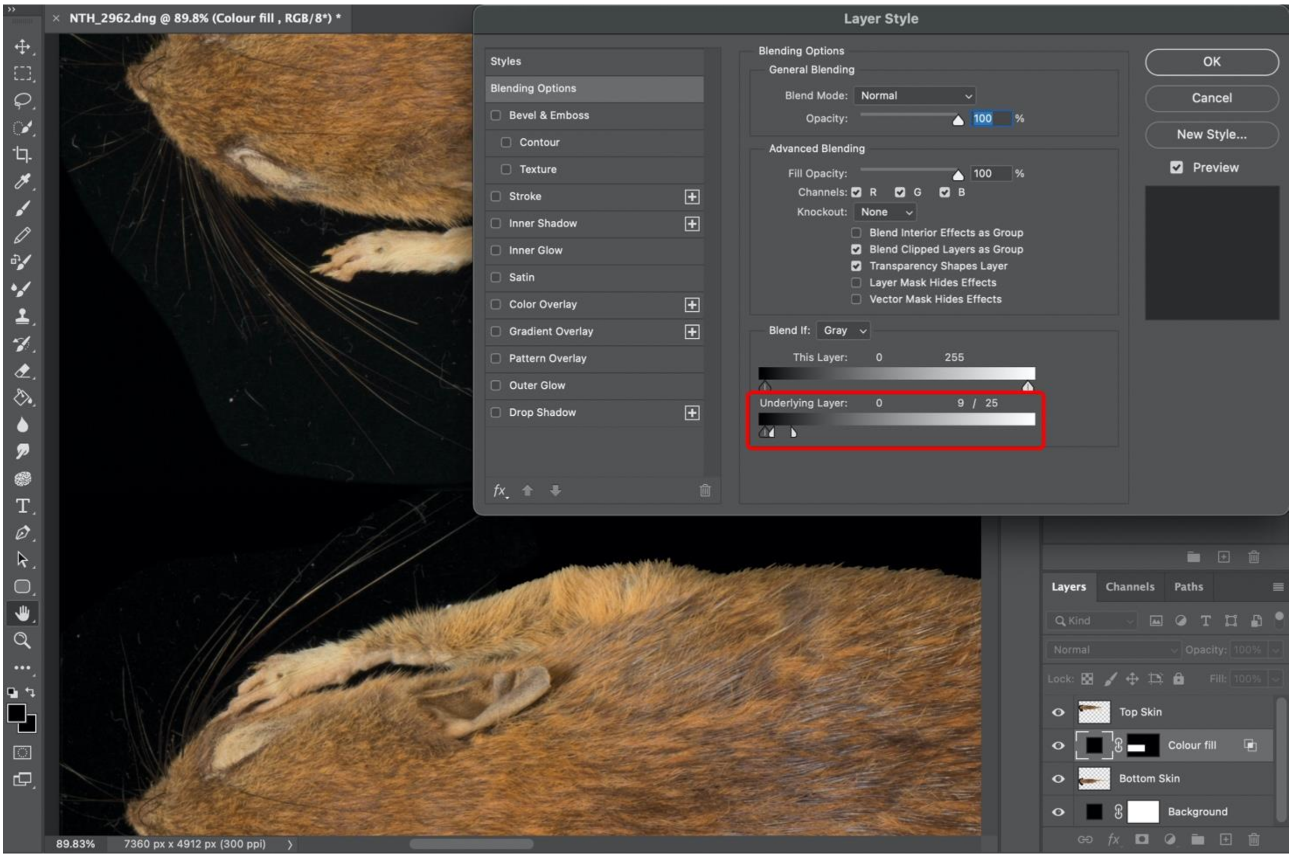

Fig. 14. Adobe Photoshop settings showing adjustments to 'Underlying Layer' option of colour fill layer blending options.

As all the steps listed above may be performed frequently, it is recommended that digitisers create 'Actions' to increase workflow efficiency. 'Actions' allow the automation of several tasks within a single command, such as commands to invert and delete selections, create scale bars with pre-defined line widths and text sizes that can be adjusted, and create colour fill backgrounds that are automatically set as the bottom layer. When creating a new action using the 'Actions' tab in Photoshop, commands and tools used are added to the action until recording is stopped.

Fig. 15. Composite image of skin of *Mus surifer leonis*, ZRC.4.3672. (Photographs by Christian Ching).

Fig. 16. Full figure plate showing paratype of *Sciurus vittatus singapurensis*, ZRC.4.3020. (Photographs by Christian Ching).

Before saving images as TIFF files, we cropped images to minimise blank spaces. To reduce file numbers, all angles of a single object or labels were condensed into individual images in Photoshop and saved as a singular TIFF file (e.g., labels, craniums, skins or mandibles). We arranged all specimen images from top to bottom in this order: dorsal, ventral and lateral (Figs. 15 and 16).

Once the composite image of a single specimen was completed, processed images were consolidated into a figure plate image file, which includes all specimen objects and aspects, as well as labels (Fig. 16). For more examples of figure plates, see the Appendix.

WORKFLOW MODULE 3: ARCHIVAL

Storage of all digitised data should ensure long-term viability of information to meet future research demands. The data stored should be easily accessible and able to be practically used, even when not available within an online data portal.

Currently, the virtual repatriation effort of the LKCNHM is still within its early stages. Due to its targeting of type specimens and with only the ZRC having been used, the number of specimens digitised is limited. The planned open-access online repository hosting metadata and images is still being designed and tested. This module will be expanded in future iterations of this publication, with updated techniques and standards for data archival.

3.1 ORIGINAL REFERENCES / DESCRIPTIONS

Original descriptions may contain information that might be unavailable during metadata collection, such as localities, associated specimens, abnormalities or other points of interest. In addition, they may inform digitisers of other associated specimens that might have been collected. We downloaded each original description of every type specimen digitised. Many of the type specimens we digitised were described within historic journals, some of which have ceased publication. We found descriptions from archival sources such as GBIF and the Biodiversity Heritage Library (BHL). We saved original descriptions of species alongside digitised metadata for ease of use.

3.2 DATA STORAGE

We archived all associated digitised data including metadata and images in both cloud and physical storage spaces. Throughout the digitisation process, we regularly backed up data (i.e., at least daily) to avoid data loss.

For digitised data of vertebrate type specimens collected from Singapore, including virtually repatriated specimens, data are archived by the NUS Libraries.

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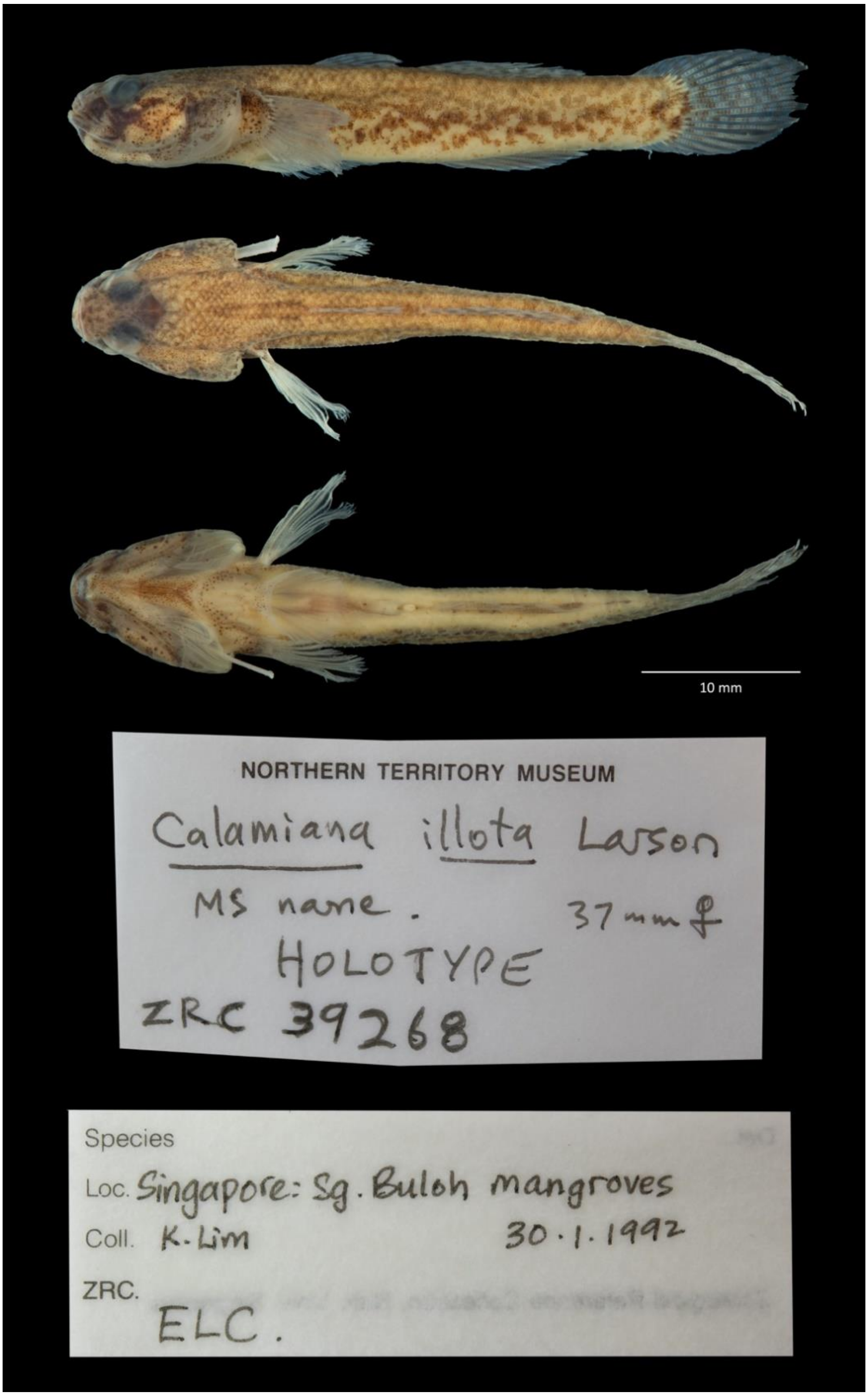
APPENDIX

Appendix 1. Selected type specimen of Actinopterygii, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum.



Calamiana illota Larson, 1999. Holotype, female, ZRC 39268, SL 37mm. Specimen locality: Singapore, Sg. Buloh mangroves. Collector: K. Lim. Date of collection: 30 January 1992. Right-side reversed. (Photographs by Shivaram Rasu & Low Bi Wei). Original description: Larson HK (1999) Allocation to *Calamiana* and redescription of the fish species *Apocryptes variegatus* and *Vaimosa mindora* (Gobioidei: Gobiidae: Gobionellinae), with description of a new species. Raffles Bulletin of Zoology, 47: 257–281.

Appendix 2. Selected type specimen of Amphibia, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum

Microhyla mantheyi Das, Yaakob & Sukumaran, 2007. Paratype, male, ZRC 1.10224, snout-vent length (SVL) 15.4 mm. Specimen locality: Sime Road Forest, Singapore. Collector: T. M. Leong. Date of collection: 11 December 2002. (Photographs by Shivaram Rasu & Low Bi Wei). Original description: Das I, Yaakob N & Sukumaran J (2007) A new species of *Microhyla* (Anura: Microhylidae) from the Malay Peninsula. Hamadryad, 31: 304–314.

Appendix 3. Selected type specimen of Aves, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum.

Gallus violaceus Kellsall, 1891. Holotype, male, ZRC 3.30131. Specimen bought in Singapore (imported from Borneo or Java). (Photographs by Christian Ching). Original description: Kelsall HJ (1891) Description of a new species of jungle fowl, said to come from Borneo. Journal of the Straits Branch of the Royal Asiatic Society, 24: 167–168.

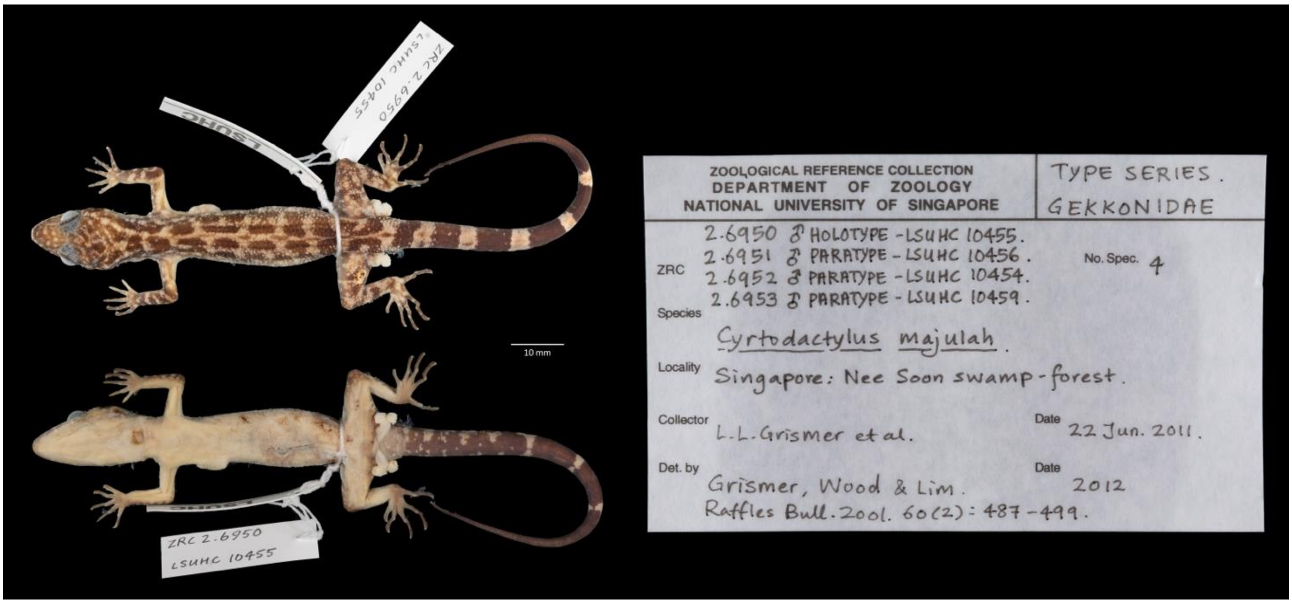
Appendix 4. Selected type specimen of Chondrichthyes, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum.

Pastinachus gracilicaudus Last & Manjaji-Matsumoto, 2010. Paratype, ZRC 50645A, disc width 302 mm, purchased at Jurong Fishery Port, Singapore. Collector: K. Lim & M. Manjaji. Date of collection: 1 February 1997. Right-side reversed. (Photographs by Shivaram Rasu & Low Bi Wei). Original description: Last P & Manjaji-Matsumoto B (2020) Description of a new stingray, *Pastinachus gracilicaudus* sp. nov. (Elasmobranchii: Myliobatiformes), based on material from the Indo-Malay Archipelago. In: Last P, White W & Pogonoski J (eds.) Descriptions of new sharks and rays from Borneo. CSIRO Marine and Atmospheric Research Paper, 32: 115–128.

Appendix 5. Selected type specimen of Mammalia, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum.

Mus surifer leonis Robinson & Kloss, 1911 [= *Maxomys surifer* (Miller, 1900)]. Paratype, male, ZRC 4.3672. Specimen locality: Changi, Singapore. Collector: H. C. Robinson & E. Seimund. Date of collection: 23 July 1908. (Photographs by Christian Ching). Original description: Robinson HC & Kloss BC (1911) On new animals from the Malay Peninsula and adjacent islands. Journal of the Federated Malay States Museums, 4: 241–246.

Appendix 6. Selected type specimen of Reptilia, collected from Singapore, in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum.



Cyrtodactylus majulah Grismer, Wood & Lim, 2012. Holotype, male, ZRC 2.6950. Specimen locality: Nee Soon Swamp Forest, Singapore. Collector: L. L. Grismer et al. Date of collection: 22 June 2011. (Photographs by Shivaram Rasu & Low Bi Wei). Original description: Grismer LL, Wood PL Jr. & Lim KKP (2012) *Cyrtodactylus majulah*, a new species of bent-toed gecko (Reptilia: Squamata: Gekkonidae) from Singapore and the Riau Archipelago. Raffles Bulletin of Zoology, 60(2): 487–499.