Modification of clinical dental impression methods to obtain whole-mouth and detailed dental traits from non-livestock vertebrates

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Running headline

Novel approach to obtain in-vivo oral data

Acknowledgments

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Author contributions

J.N.W., R.S., T.K., and L.S. conceived the project. J.N.W. and R.S. conceived the method and performed overall direction and planning. J.N.W., T.K., and R.S., performed molding trials. J.N.W. designed and P.P. parameterized and visualized the impression tray. T.K. and R.S. performed microscope scanning of obtained molds. T.O. performed morphometrics analysis. J.N.W., T.K., R.S. and L.S. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our research integrates authors from different countries, including the country where the study was conducted.

Data availability

Parameterized blender file and STL files for the impression trays used in this project are available on https://anonymous.4open.science/r/impressiontray-24FE/README.md and is archived on Zenodo (doi:10.5281/zenodo.12524788).

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Ethics statement

This research was approved by the ethics committee of the Okinawa Institute of Science and Technology under the protocol number ACUP-2023-006-2.

Competing interests

The authors declare no competing interests.

Abstract

1. Dental impressions, important for capturing oral characteristics in clinical settings, are seldom used in research on non-livestock and non-mammalian vertebrates. Live animal dentition studies usually require euthanasia and specimen dissection, microCT and other scans with size and resolution tradeoffs, and/or ad-hoc individual impressions or removal of single teeth. These practices prevent in-vivo longitudinal studies that factor in growth and other chronological changes, limit the ability to capture and compare data from a range of species, and separate teeth from the context of the whole mouth and soft tissue.
2. Here, we describe a non-destructive, humane method for obtaining high resolution, whole mouth and dentition traits that can be used on both living animals and museum specimens, involving a novel printable dental impression tray that is highly customizable in terms of size and shape.
3. We demonstrate that this method can repeatedly and accurately capture whole-mouth morphology and detailed features at high resolution using living *Polypterus senegalus*, a non-teleost fish (Actinopterygii), in a laboratory setting.
4. This method can be used with all vertebrates, including those without commercially-available veterinary dental impression trays. In living animals, it can be used for comparative morphology and to observe temporal changes such as microwear, tooth replacement rates, and bite and morphological changes through ontogeny.

Keywords: dentition, in vivo dental impression, oral morphology, Polypterus, comparative anatomy, dental microwear

1 Introduction

Vertebrate dentition provides essential information on the ecology and evolution of species. In fishes alone, dental traits have been used to investigate wide-ranging topics such as development (Hung et al., 2015), replacement (Carr et al., 2021), dental microwear analysis (Purnell and Darras, 2015), jaw evolution (Ronco et al., 2021), feeding characteristics (Streit et al., 2015), biomechanics and bite force (Deng et al., 2022) and diversification of the oral apparatus (Burress, 2016). However, all these studies used either dead specimen or isolated teeth (Burress, 2016; Streit et al., 2015), and euthanasia was often necessary to obtain samples (Hung et al., 2015; Ronco et al., 2021). Furthermore, dissection of the jaw and/or removal of teeth is typically performed, damaging the original sample. The same constraints limit *in vivo* studies of other vertebrate dentitions and oral traits, apart from those on livestock and a handful of primates for whom clinical dental practices exist (Hoffman et al., 2015; Teaford and Oyen, 1989).

In order to avoid destructive practices in obtaining morphological data, scanning methods (e.g. optical, microCT, laser, and Synchrotron) have been increasingly used on full specimens (Akhter and Recker, 2021). However, scanning approaches also have constraints which limit their utility in many vertebrates. Optical scanning, as of 2024, lacks the resolution necessary to see fine details such as wear (L.S. and T. K. personal observation). MicroCT and Synchrotron X-ray scanning imposes size limitations on specimens and have trade-offs between field of view, processing time, and image resolution, and of course take place under high radiation (Sun et al.,

2022). Usage of X-ray scanning therefore requires dead and or dissected samples in most cases and/or considerable preparations and planning at limited facilities for limited *in vivo* work to date (Gradl et al., 2018).

Here, we propose and demonstrate a noninvasive alternative methodology to obtain high resolution dental and oral trait information. Based on human clinical practice, we designed 3D printed, adjustable dental impression trays which can be used with readily available, inexpensive non-toxic impression materials to take multiple whole intraoral records from living and dead vertebrates. Direct application of dental impression material to non-human teeth has been shown to accurately capture fine surface details (Purnell and Darras, 2015; Sawaura et al., 2022). However, this ad-hoc application was limited to fixed dead and skeletal animal specimens or fallen teeth. The use of a species-customized tray allows for repeated use with live animals, for tracking of chronological changes, and exact capture of whole mouth morphology.

Here, we demonstrate the utility of our modifiable, freely printable tray in taking detailed oral impressions from non-human vertebrates (see below and supplementary information for download and adjustment details). We developed and tested this methodology on live and preserved specimens of a non-model but increasingly used laboratory organism, the non-teleost fish *Polypterus senegalus*. Below, we describe the details of our tray, its proper usage, and the results obtained by this method.

2 Materials and methods

2.1 Impression tray design

A dual arch impression tray system (Figure 1A) with a handle was chosen as the basis for our design, eliminating the need for each animal to undergo multiple sessions to take impressions for both maxillary/upper and mandibular/lower arches. Tray design was built to include intraoral and extraoral components based on trials with live fishes (Supplementary Material) and human dental tray designs. We placed a crescent-shaped trench of 0.5-1 mm in depth and of uniform on both maxillary and mandibular aspects of the tray to allow extra occlusal clearance for dentition to impress the material (Figure 1A).

An extraoral perforated retaining wall in front of the trench allows for firm stabilization during impression sequences (Figure 1C-F), as movement by the animal or the operator would otherwise negatively affect accuracy and material distortion. Our wall element extends posteriorly and past to the angle of the mouth in order to affix and stabilize the head. Perforations were included in the design of the retaining wall to aid in attaching the impression material to the tray without the use of adhesives. The tray wall can be customized to match the external border and size of the jaw (Figure 1A, right). The intraoral portion of the tray includes occlusal stops to avoid tooth contact with tray during impression and prevent posterior sliding (Figure 1A, middle). Incorporating stops based on oral morphology ensures a uniform thickness (Terry et al., 2010) and minimizes distortion (Ortensi and Strocchi, 2000). In our *Polypterus* test animals, the centrally located parasphenoid bone on the oral roof and the “tongue” (basihyal) in the mandibular floor were designated as hard/soft tissue stops. We inserted a centralized raised stop behind the trench on maxillary section of the

tray and a smaller raised central lingual floor on the mandibular side in our test trays, both of which can be removed before printing (Supplementary Information).

The final version of our adjustable, 3D-printable impression trays was designed using Autodesk Fusion360 and parameterized using Blender 4.0. Inclusion of sliders and checkboxes in Blender to adjust the size, relative dimensions, and intraoral and extraoral features (stops, holders, etc.) enables application to a wide range of mouth shapes and allows the tray be modified without technical skills in 3D modeling. The model is freely available and archived on Zenodo (doi: 10.5281/zenodo.12524788). The steps to modify the tray is outlined in supplementary figure 1.

2.2 Intraoral impression process

The following protocol was developed through testing with live and preserved *Polypterus sp.* Details of fish husbandry, sedation, and other specific aspects can be found in the Supplementary Information. This protocol can be modified for other species and to use equivalent impression materials. We recommend consultation with a veterinarian, Animal Care and Use Committee, and/or published, established protocols to determine appropriate husbandry and sedation of other species.

Adjusting and printing impression trays

We first adjust our tray models using Blender to the specifications for species and individuals. We then 3D print trays using an Ultimaker S5 (Ultimaker, Netherlands) with Ultimaker PLA material (Ultimaker, Netherlands), but any printer can be used with PLA material from any source. Ultimaker Cura is used to slice the STL files generated from Blender, using the default preset “extra fine” with 0.06 mm layer height. We find that the tray prints best with the support overhang angle at 80° and brim build plate adhesion type, printing the structure upright with the handle on top

and the mouthpiece at the bottom. Less Z axis resolution can be used with larger impression trays.

First impression to remove debris

After preparation (Figure 2A) and confirming the animal has been completely sedated if alive and not domesticated (Figure 2B; Supplementary Material), tweezers are used to gently pry the mouth open to expose the teeth. Compressed air is used to dry the dentition to remove residual moisture (Figure 2C). First impression is taken with Vinyl Polysiloxane Putty Type (we used ExaFine, GC, Japan), which is mixed according to manufacturer directions and then directly applied to dentition to remove and reduce enamel pellicle (Figure 2D-E). Gentle pressure is used to close the mouth on the putty material 2-3 times ensuring that the teeth are fully submerged while the putty is relatively soft. Putty is removed in about 1 minute prior to complete hardening to reduce the risk of enamel damage.

Final impression sequence

To prepare for the final impression using a more accurate impression material, an Automix tip is affixed to a Polyvinyl siloxane (PVS) cartridge (we used Dr. Silicon regular, BSA Sakurai, Japan) to ensure proper mixing of impression components. This material has been shown to cure quickly and has high accuracy (Sawaura et al., 2022), suitable for a wide range of applications including dental microwear study, and is routinely available from dental suppliers (Supplementary table 1 and 2). A portion of the impression material is exuded from the tip prior to filling the impression tray to ensure that only properly mixed material is injected.To avoid air pockets, the impression dispenser Automix tip remains submerged inside the impression material as it is ejected into the tray.

The loaded impression tray is placed into the mouth of the animal, where both jaws were pressed together to simulate a bite (Figure 2G). Animal and impression tray are held together securely yet gently to avoid harm until the impression material was completely set (Figure 2H). Curing time was set to 5 minutes or more to let the material harden (Sawaura et al., 2022), but this may vary with PVS from other manufacturers. The live animal is then returned to its original confines and monitored until it regains motor functions. This two-impression process takes approximately 10 minutes.

3. Proof of concept results and discussion

3.1 Trait resolution (Based on *Polypterus)*

Using this method, we were able to produce impressions that accurately captured dentition and intraoral morphology of very small live specimens of *Polypterus* (Figure 3A-B).). We scanned the impressions taken from different individuals (see Supplementary Material for details of scanning and image production) and effectively captured dental structures less than 1mm across the entire jaw (Figure 3C-D and supplementary figure 2A-B), a higher resolution than a previous study using direct manual measurements (Mihalitsis and Bellwood, 2019). Further, we identified enamel damage on some teeth by quantifying the depths of the ridges on the surface of the teeth (Supplementary figure 2B-C). From 38 impression attempts on 12 individuals, there was no incident of specimen mortality or injury due to the impression process.

3.2 Reproducibility

As this is a qualitative method, we tried to assess the reproducibility and accuracy of obtaining shape information through geometric morphometric analysis of maxillary/upper impressions (Figure 3E-F, Supplementary figure 3-4). This allowed quantification of impression method error, landmark assignment operator error, and individual variation, based on generating 5 sets of landmarks for the parasphenoid and associated soft tissues in each of 4 maxillary impressions on each of 2 different, very small (approximately 20cm) individuals in one sedation session (40 total landmark sets) (Supplementary figure 3). We performed principal component analysis (PCA) (Figure 3E, Supplementary figure B4-C), and found that PC1 was associated with differences between individuals, while PC2 was associated with some impression method error (the difference between a tight clustering of most impressions and an outlier). Outliers were not related to order of impression and did not include any initial impressions. Using the same parasphenoid landmarks, we also conducted a geometric morphometrics analysis of all available whole maxillary impressions from 7 individuals taken over multiple sessions, spread over the multi-month course of method development and data collection for another project (Figure 4F, Supplementary figure 4D-E). Our PCA results for all individuals further demonstrated that variation between different individuals, or the same individual overgrowth, was much greater than any method error or operator error generated in a single session. This suggests that this method can quantify the gross oral morphology of individual specimens reproducibly, and capture variation over time within individuals and across populations.

We calculated disparity values for landmark sets from duplicate images of single impressions, multiple sequential impressions of one individual, and from all 7 study

individuals (Supplementary figure 4F). Levels of variance between duplicate images of one impression were comparable, representing similar levels of operator error between observations. Disparity was highest for all impressions from individuals (reflective of individual variation) and lower for multiple impressions from one individual even including outliers on PC2 (Figure 3E), reflective of method error and not the sequence in which impressions were taken. This indicates the method can typically accurately detect and represent distinctions between individual variations with high fidelity and a low rate of error in shape.

3.3 Potential applications

As above, this method can be used to non-destructively and cheaply obtain whole mouth oral traits at high resolution from both live and dead specimens. Therefore, it can be used for any morphological studies involving oral traits, occlusion, and dentition in place of existing methods that are destructive (e.g. dissection), expensive (e.g. microCT), and/or provide high resolution for a much smaller area (e.g. Synchrotron, SEM), as well as methods with less control (e.g. direct application of putty), or reduced resolution (e.g, optical scanning). One of the most important benefits of the new method is that it allows production of time series for morphological changes across the entire dentition and oral-pharyngeal cavity of living animals. This is useful for studies of dental microwear analysis (Hoffman et al., 2015) as well as growth, replacement, and development (Carr et al., 2021; Streelman et al., 2003; Trapani et al., 2005). Dental impressions can also record detailed intraoral soft tissue anatomy of living specimens, including features previously could only be evaluated on dead specimens (Luczkovich et al., 1995).

Some possible applications of this tray-mediated impression method include the production of physical and 3D digital models from live or rare specimens which cannot be prepared for high resolution scans by microCT or other means. Impressions can be used for geometric morphometric analyses of dentitions in a range of species. Occlusion patterns preserved in impressions can provide an alternative approach to study bite articulation and jaw biomechanics (Wilga and Ferry, 2015). Soft tissue and high resolution dental models created from scans of impressions can also be of use with the physical replication of skeletal movements and feeding mechanics for (XROMM) studies of individuals, which typically depend on whole body CT scans at low resolution (Camp and Brainerd, 2015). Although tooth replacement has been extensively studied from a histological and developmental perspective there is very little data on tooth growth and replacement on living non-mammal individuals (Carr et al., 2021), especially with time series from a single individual. Recurring creation of whole-dentition tooth records via dental impression provides an opportunity to not only study replacement patterns but also examine external factors that can affect it. We are positive that researchers focused on the ecology and evolution of vertebrates will find many additional uses for the data captured by our method. The considerations for limitations of this method are discussed in the supplementary materials.

3.5 Material sourcing and cost considerations

Costs are reduced by using existing, consumer-grade, and readily available 3D printers and material. In principle, most rigid 3D printing materials can be used, here we opted to use poly-lactic acid as it is one of the most available and cheap material.

Dental impression compounds can also be selected based on the purpose. For fish, the use of MS-222 anesthetic can be costly, thus clove oil can be used as a substitute due to its potency and cost (Javahery et al., 2012). The same anesthesia mix can also be reused for multiple aquatic individuals, as it is mediated by water. The breakdown of the materials and costs for our tests are available in supplementary table 1 and 2.

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Figure legends

Figure 1. Schematic Representation of the Impression Tray and Usage

Schematic representation of the dental impression tray and impression process, using *Polypterus* as an example. (A) 3D rendered image of the impression tray showing mandibular/lower side, maxillary/upper side, and lateral view. (B) Impression trays in multiple different sizes. (C-D) Impression material is applied to both sides of the impression tray. (E-F) *Polypterus* biting on the impression material.

Figure 2. Example Impression Sequence Using *Polypterus*

Molding sequence of *Polypterus*. (A) Preparation of materials before starting the procedure (B) Individual in buffered MS-222 bath. (C) Removal of residual moisture using compressed air duster. (D-E) Removal of impurities from teeth surface using putty. (F) Application of PVS material on the surface of the impression tray. (G-H) Molding of the teeth by firmly pushing the jaw onto the impression tray.

Figure 3. Example impressions from *Polypterus*

PVS mold obtained upon the completion of impressioning. (A-B) Undetached impression from (A) maxillary side and (B) mandibular side. (C-D) Lingual scans of impression obtained from the maxillary side for (C) posterior tooth and (D) anterior tooth. The size of photo-simulations (C-D) is 100 micrometers (Y axis) times 140.5 micrometers (X axis). (E) PCA plot of morphometrics analysis using landmarks from 4 maxillary/upper impressions of each of 2 individuals. 5 landmark sets were digitized from each impression (represented by gray convex hulls). and (F) from 7 individuals. (G) PCA plot of geometrics morphometrics analysis of all available whole

mouth impressions (N=13) from 7 individuals used in method development. Some impressions were omitted when they were deemed to be deformed due to contact between animal and tray (eliminating putty) and could not be landmarked, or when they were cut to obtain details on tooth impressions featured in Fig. 3A.

Supplementary Figure 1. Tray customization

Customization of the impression tray using Blender. (A) The supplementary (or downloadable) file parametric_tray.blend can be loaded into Blender. (B) Relative parameters can be adjusted depending on the target species by adjusting the values or the slider on the pane on the right side of the window. (C) Perforations need to be cut after adjustments and before exporting to STL as it is a computationally intensive process. (D) STL can be exported by selecting export > Stl (.stl).

Supplementary Figure 2. 3D scanning of tooth impression

(A) the mandibular side for posterior tooth. (B-C) Lingual scan of the mandibular side for a middle tooth, which enamel was flaked off by brushing, (B) photo-simulation and (C) topology showing the elevation at the area where enamel was flaked off. The size of photo-simulations (A-B) is 100 micrometers (Y axis) times 140.5 micrometers (X axis).

Supplementary Figure 3. Impression taken from two individuals for morphometrics analysis

Impressions obtained from 2 different individuals in single sedation sessions (~20 minutes total). (A-D) Impressions taken from individual 1. (E-H) Impressions taken from individual 2.

Supplementary Figure 4. Geometric morphometrics analysis

Geometric morphometrics analyses. (A) Landmarks used to perform morphometrics analysis. 42 landmarks were placed in total; white numbers are fixed landmarks, and red numbers are semi-landmarked curves. (B-C) PCA plot (N=40) showing (B) PC1 and PC2 and (C) PC3 and PC4 from the morphometrics analysis of 2 individuals. Each impression is represented by the grey convex hull. (D-E) PCA plot (N=45) showing (D) PC1 and PC2 and (E) PC3 and PC4 from the morphometrics analysis of 7 different individuals. (F) Results of disparity calculations for image duplicates and impression duplicates. "Overall" disparity shows procrustes variance across impressions taken from individuals 1-7. "Method" disparity shows procrustes variance between all impressions taken from specimen 1 and specimen 2 respectively. "Operator" disparity shows the difference between all duplicated images from each impression used in the principal components analysis in figure 3E and supplementary figure 4B and represents operator error.

Supplementary Table 1. Impression material cost

The approximate cost of impression material for the experiments performed in this study.

Supplementary Table 2. Alternative impression material cost

The approximate alternative cost for impression materials with a more cost-effective option. Alternative materials can also be used when performing experiments on larger fishes that require higher anesthesia dose.

Figure 1

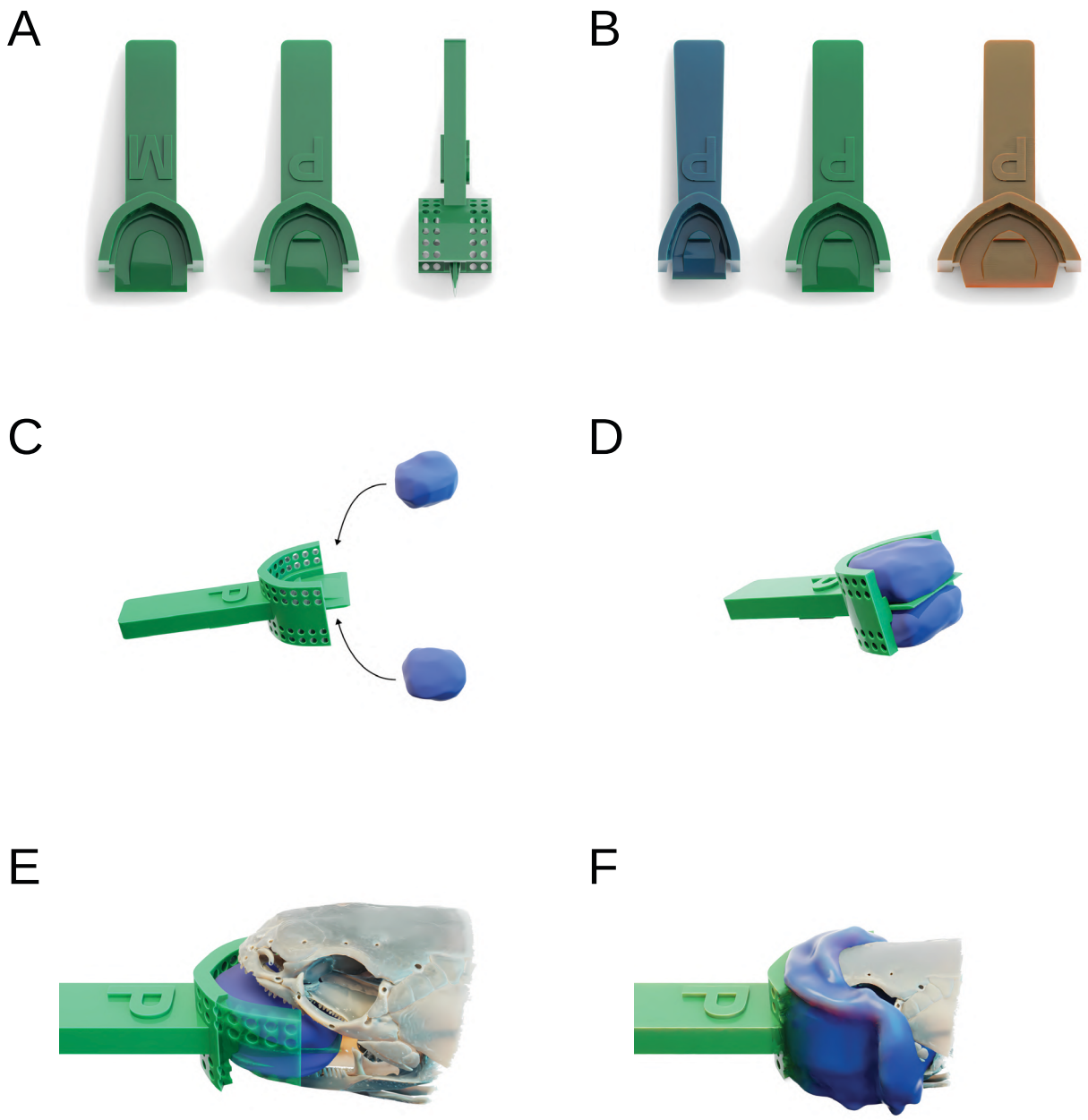
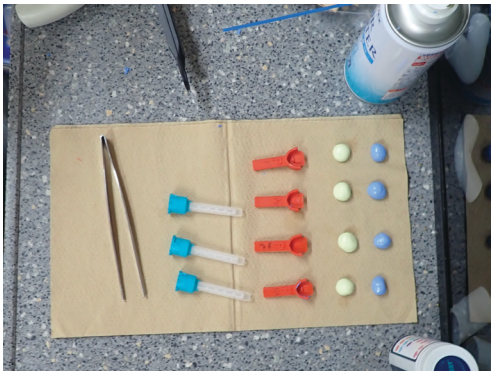


Figure 2

A



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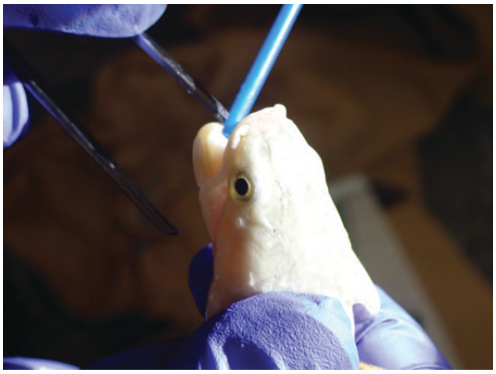

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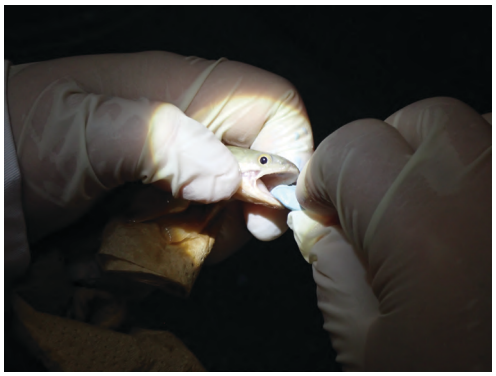

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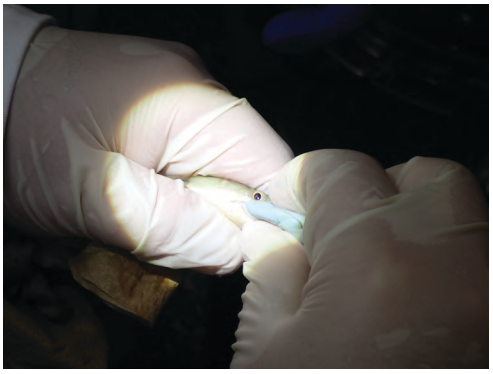


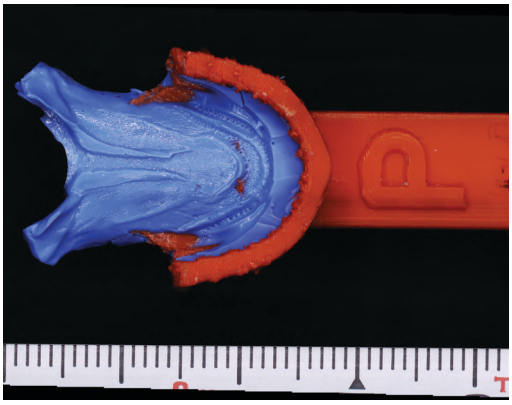
F

G

H

Figure 3

A



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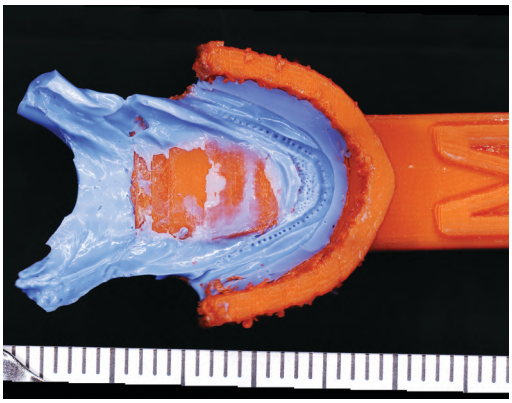

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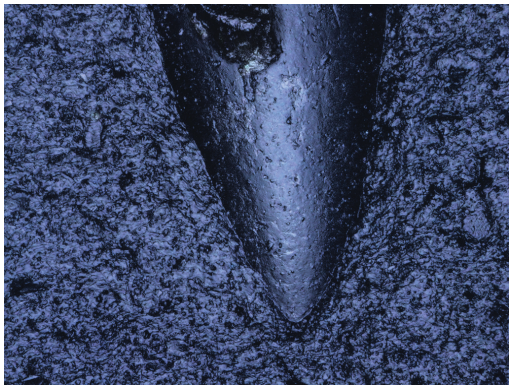

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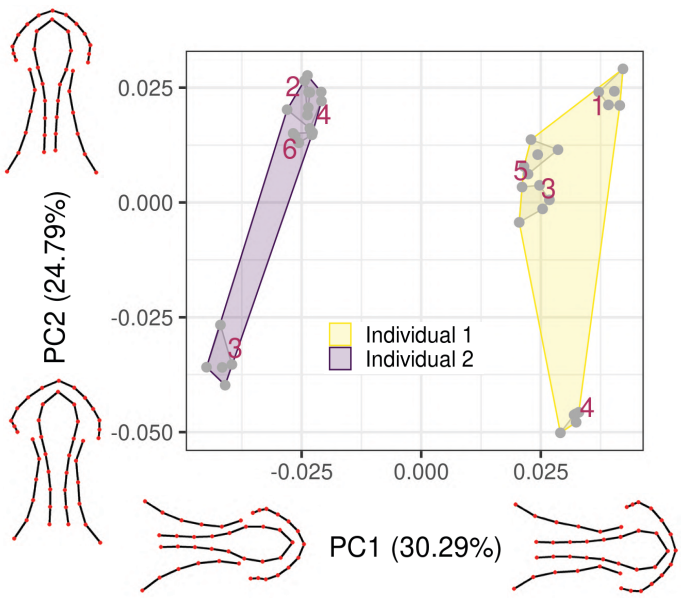

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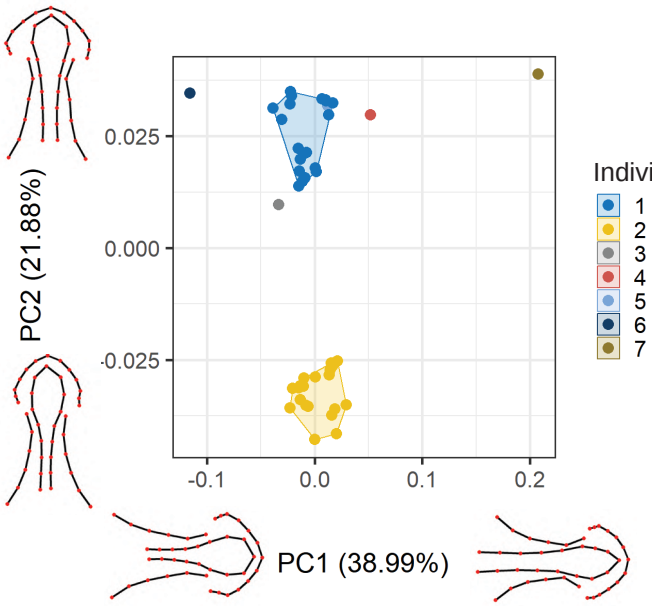

Supplementary figure 1

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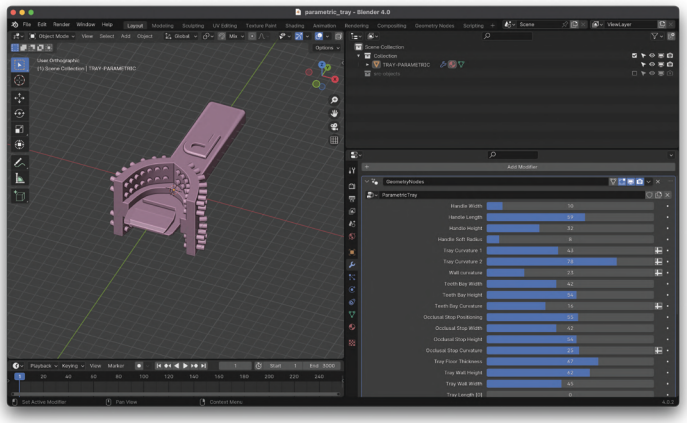

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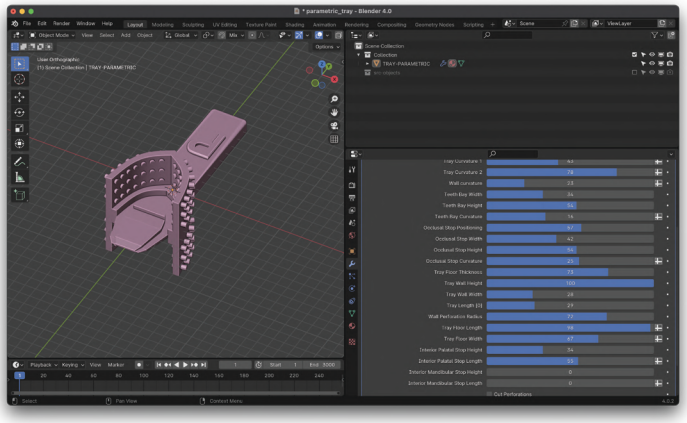

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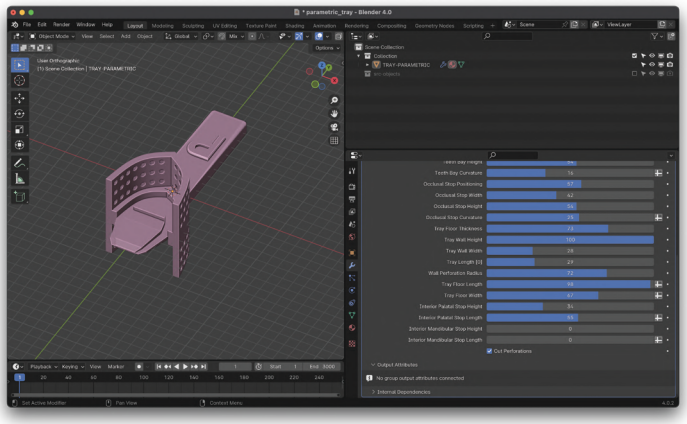

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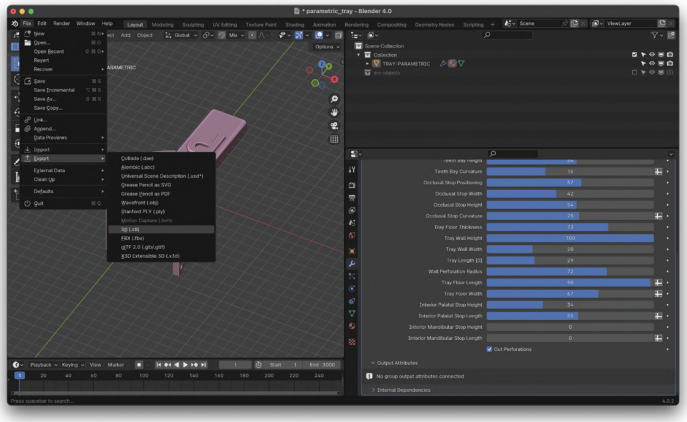

Supplementary figure 2

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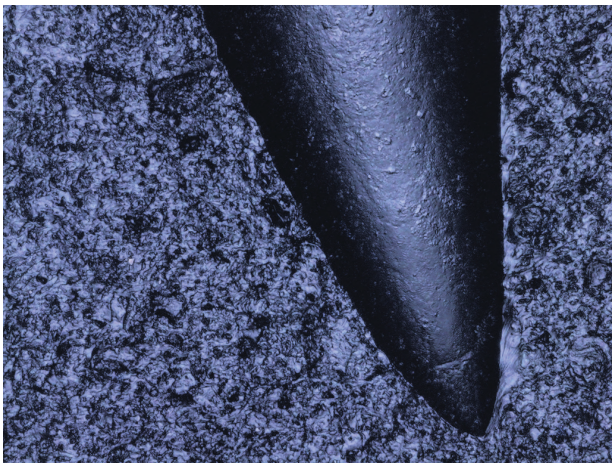

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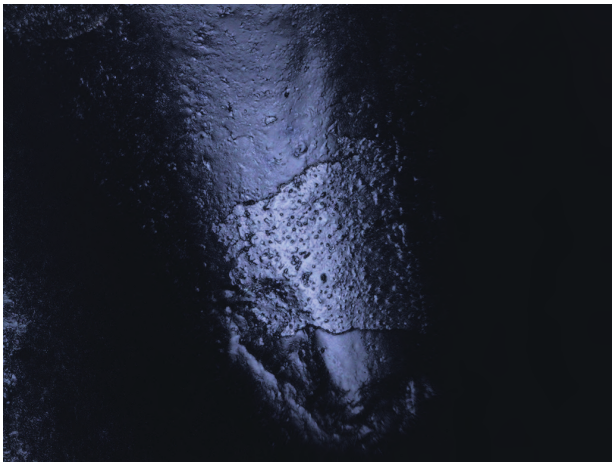

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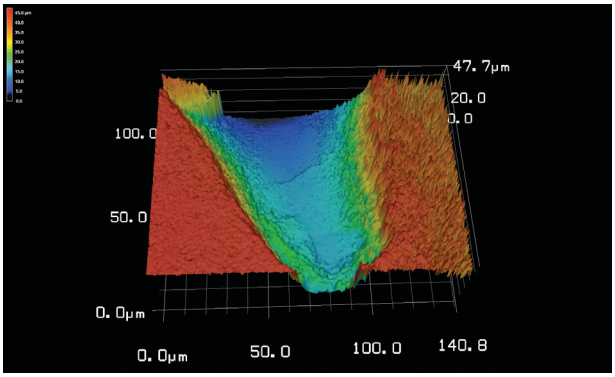

Supplementary figure 3

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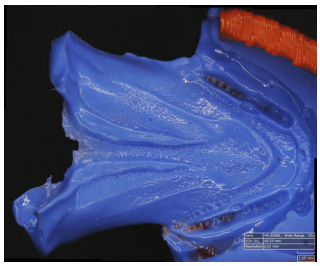

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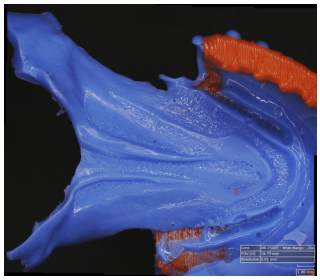

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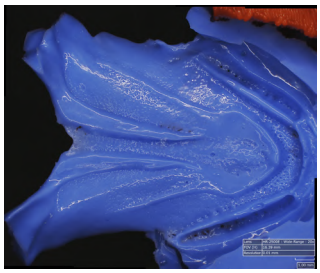

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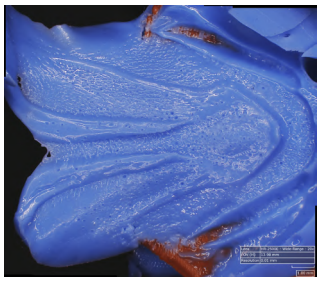

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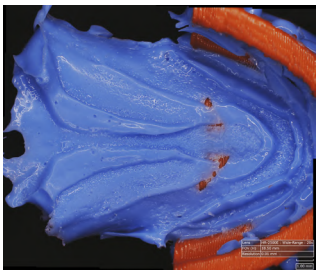

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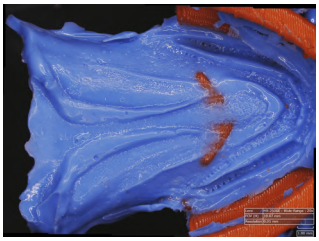

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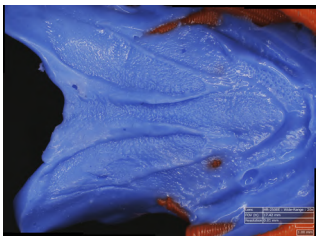

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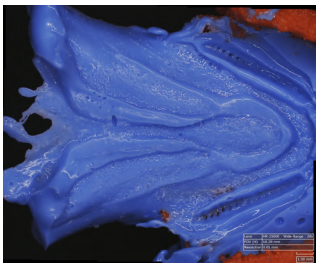
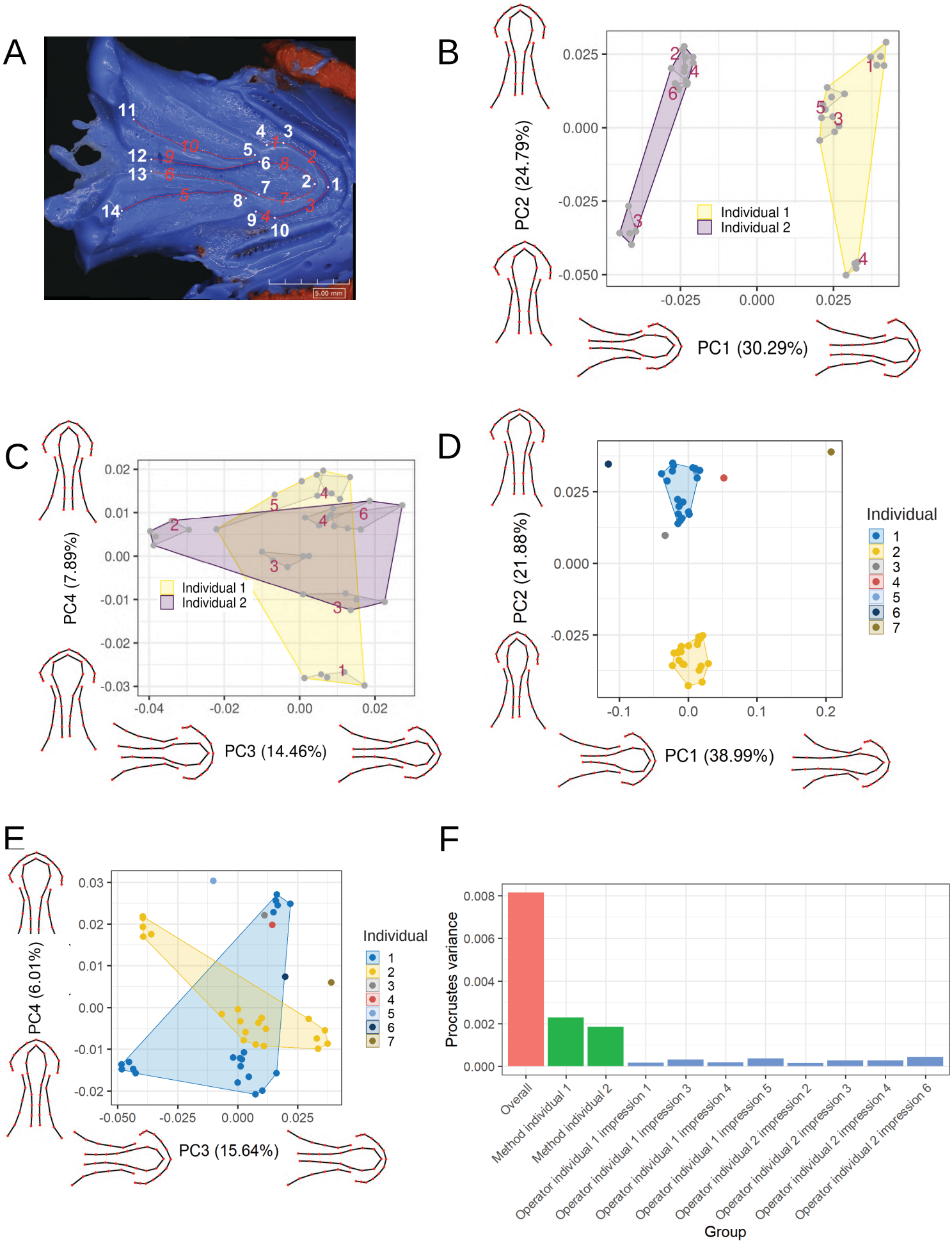


Supplementary figure 4



Supplementary table 1

Type	Item	Manufacturer	Approximate cost in USD	Use per experiment	Approximate cost per experiment in USD	Note
Impression material	Dr. Silicone regular type (medium body) 50ml cartridges	BSA sakurai	10 per cartridge	~5ml per animal	1	Tray size 2 cm by 2 cm deep and included filling palatal and mandibular sides.
Impression material	Exifone putty 556 ml (combined base and catalyst)	GC Japan	60	2.8 ml per animal	0.3	A quarter of a mix of 1 scoop of base and 1 scoop of catalyst.
Anesthesia	Ethyl m-Aminobenzoate Methanesulfonate (MS-222) 25g	Nacalai Tesque	108	0.6g in 2L of water	2.6	Anesthetic mixed water can be used for multiple fish cohabiting the same tank.
3D printing	UltiMaker NFC PLA Filament 2.85mm (750g)	Ulti Maker	50	1g	0.1	Autoclavable trays can be reused on different animals.

Supplementary table 2

Type	Item	Manufacturer	Approximate cost in USD	Use per experiment	Approximate cost per experiment in USD	Note
Anesthesia	Clove oil C8392-100ML	Sigma Aldrich	55	Approximately less than 0.2ml	0.1	We assume that ethanol that is used to dissolve the clove oil is already present in most labs
3D printing	Generic PLA 1kg	various	20-30	1g	less than 0.1	
3D printing	Generic ABS 1kg	various	20-30	1g	less than 0.1	
3D printing	Generic PETG 1kg	various	20-30	1g	less than 0.1	
3D printing service	3D printing service	various	1-2	-	1-2	