How many species are there in Lake Kivu?

A geometric morphometric approach to reveal the species diversity of the *Haplochromis* (Cichlidae, Teleostei) genus in Lake Kivu.

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The *Haplochromis* assemblage of Lake Kivu has always been considered as very species poor. Within the scope of my Bachelor Thesis I examined a collection of Lake Kivu cichlids to find evidence for greater species diversity than previously known, using a geometric morphometric approach. I show here that the nine *Haplochromis* species included in our collections that were know from Lake Kivu previously to this study represent the most extreme morphologies, and morphospace did not increase when adding putative new species. Instead Mahalanobis distances between groups decreased strongly, suggesting that putative new species fall within the large morphological gaps that lie between previously described *Haplochromis* species. I also tested for morphological shifts along the depth gradient, as little is known about community composition at different depths in this lake. These analyses show that species with deeper bodies and short snouts, typical "algae scraper" morphology, are restricted to shallow water, whereas species that show a more intermediate phenotype often have a very wide distribution along the depth gradient.

Introduction

The African cichlid fish radiations are the most diverse vertebrate adaptive radiations known (Seehausen, 2006). More than 2000 African cichlid species evolved within the past five to six million years (Turner, 2001). Although cichlids have radiated in many lakes in Africa, most research has concentrated on the most diverse radiations in the East Africa Great Lakes, Malawi, Tanganyika and Victoria. Smaller radiations have often been overlooked (Seehausen, 2006).

One such radiation is found in Lake Kivu. Lake Kivu is located 120km north of Lake Tanganyika in the Albertine Rift and is connected with it via the Rusizi River. With an area of 2'700 km^2, it is about 5 times the size of Lake Constance (Switzerland/Germany). Nevertheless, since the first scientific collection by J.E.S. Moore during his second Tanganyika expedition (1899-1900), Lake Kivu was always described as having a species-poor fish fauna (Snoeks, 1994). Only 15 endemic haplochromine species are described in the latest report concerning its overall ichthyological diversity (Snoeks, 2012).

Within the scope of my bachelor thesis I want to determine if there is morphological evidence for greater species diversity than the 15 currently described haplochromine species in Lake Kivu. To examine morphological diversity in the haplochromines of the Lake Kivu radiation I used landmark based geometric morphometric methods. These

methods are powerful in discriminating even closely related species morphologically (Zelditch, 2004, Klingenberg et al 2003).

I used multivariate methods to examine morphometric patterns and clustering in cichlid collections from Lake Kivu, and calculated Mahalanobis distances between species preliminarily identified based on morphology. I also looked at differences in morphospace related to the depth at which specimens were caught to assess shifts in ecology, expressed in shape, of the haplochromine assemblages as depth increases.

Methods

I analysed fish collected in the Rwandan territory of Lake Kivu in April 2012. Because fish collections were made for the purpose of generating baseline fish biodiversity estimates for Lake Kivu prior to the instalment of the KivuWatt Methane extraction platform, sampling was restricted to the area around Kibuye Bay on the Eastern shore of Lake Kivu.

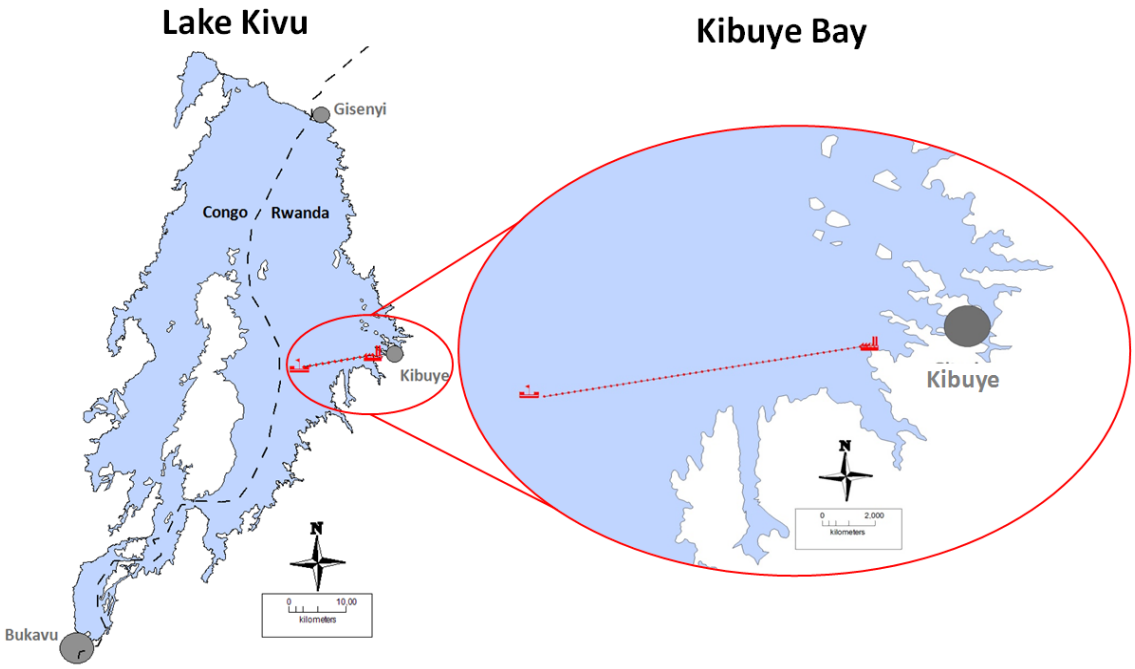

Fig. 1: Map showing the study area and the location of the planned KivuWatt platform as well as the onshore facilities. Sampling area is located east to the platform. (Modified from Paris et al. 2013).

A combination of a stratified random sampling method (European method CEN), and a habitat-targeted sampling (Vertical Net Method: Degiorgi et al., 1994) was applied in both the pelagic and littoral zone. In the stratified sampling method multi-mesh gillnets were set at randomly chosen locations in pre-defined depth ranges to assess fish diversity along the lake's depth gradient. In the habitat-targeted sampling approach the littoral habitat within the study area was first mapped until a depth of 6 meters using GSI based software. Habitat that was deeper than 6 m was always called demersal. Each habitat was sampled at least 3 times at different locations in the study area. This second sampling approach is an important complement to the stratified random sampling as it aims to catch species that are restricted to a certain habitat and are not randomly distributed over the lake.

Gill nets were set to 75m but no fish were expected lower than 35-50m, as below this depth the water contains no oxygen. In the field season of April 2012 the oxycline was located at 36m and no fish were caught below 35m depth.

The identification of species was done in field with the help of African freshwater fish taxonomic literature (Snoeks, 1994; Seehausen, 1996), and by O. Seehausen based on standardized colour photographs of live fish. Out of 584 fish O. Seehausen assigned 150 individuals to 9 existing species and 22 putative new species. All fish were then fin clipped, tagged and preserved in formalin. Fin clips were preserved in 75% ethanol for future genetic analyses. All fish and fin clips are vouchered in collections at EAWAG.

I first took standardized photographs of all haplochromine cichlids in the collections made from Lake Kivu in April 2012. To do this, the fishes were put on a white plastic board as background and pinned down in such a way that all 17 defined landmarks were clearly visible on the photograph. All photographs were taken with the digital reflex camera Canon D60 with a Canon fixed focal length lens (35mm). The camera was mounted on a stand, focus was adjusted manually and photos were taken with slight over-exposure in order to enhance visibility of details on the often dark fish.
Lens distortion was measured by photographing a ruler on a grid from different heights and measuring grid dimensions afterwards on the computer with ImageJ (Rasband, 1997). Lens distortion was found to be negligible from all heights.

17 predefined landmarks were placed on each picture using the TPSdig software (version 2.16) (Rohlf, 2006). With the exception of landmark 17, all landmarks followed those of Selz et al. (2013). I revised all photographs for misplaced landmarks the day after initially placing the landmarks. To estimated error in landmarking I performed a procrustes ANOVA. I randomly chose 7 individuals and landmarked each twice to check the deviation of landmarks between individuals generated by error in landmark placement. This is a measure of how much of the difference of landmark coordinates is really due to differences in shape of different individuals versus error in setting the landmarks. Error due to flawed landmarking turned out to be negligible ($F_{7,8}$=2086.27, p<0.0001)

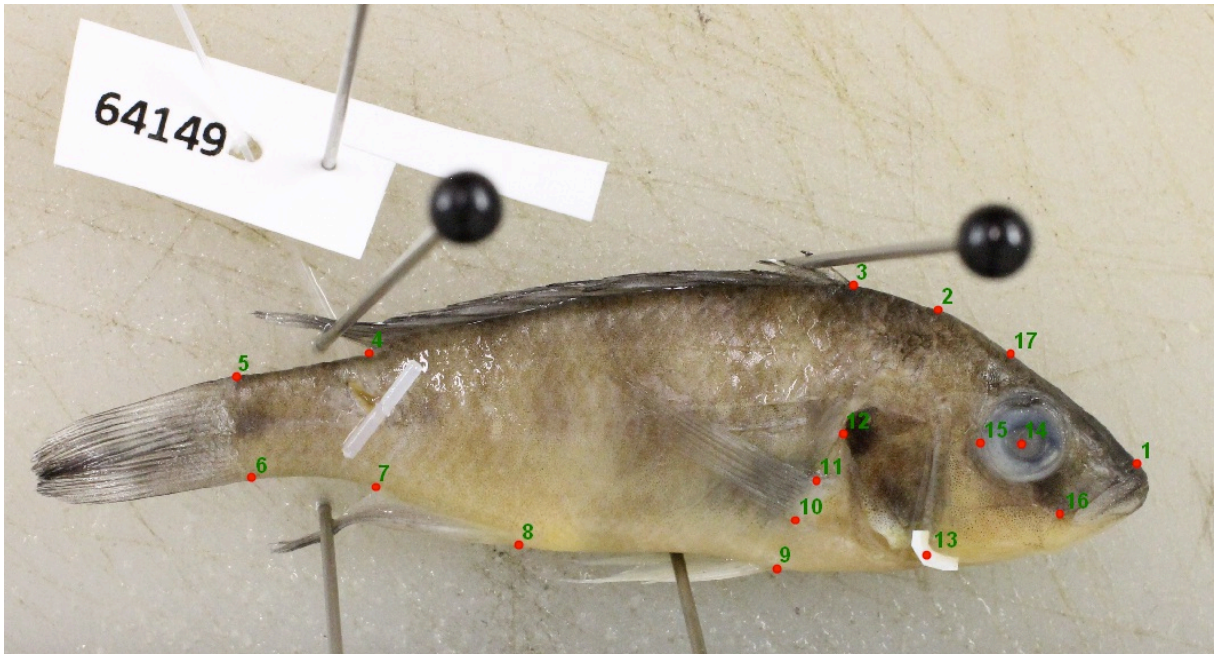

Fig. 2: 17 landmarks were set on the photographed fish as described in Table 1.

Table 1: Description of the individual landmarks

1	Landmark is set to where skull meets maxilla, not on the maxilla itself.
2	Where head and body scales meet.
3	Where the first ray of the dorsal fin inserts.
4	Where last ray of the dorsal fin inserts.
5	Where larger body scales turn into smaller scales near the caudal fin. Often these larger scales form an edge with the smaller ones and it seems like this is the point where caudal fin gets bent when the fish is swimming.
6	Same as landmark 5 but ventral.
7	Where the most posterior ray of the anal fin inserts.
8	Where the most anterior ray on the anal fin inserts.
9	Where the most anterior ray of the pelvic fin inserts.
10	Where the most ventral ray of the pectoral fin inserts the fleshy part of the pectoral fin, which then inserts the body of the fish.
11	Same as 10 but dorsal.
12	Most posterior part of the operculum.
13	Most posterior-ventral point of the preoperculum.
14	Middle of the eye. Not the middle of the pupil.
15	Anterior reach of the eye. Draw a line between landmark 1 and middle of the caudal fin. Landmark 15 and 14 should then be parallel to this line.
16	landmark not on the most posterior point of the maxilla but on the grove in which the dorsal posterior end of the maxilla rests when the mouth is closed.
17	In the middle of landmark 1 and 3 and not as in done by Selz, 2013 in the middle between landmark 1 and 2. I have the impression that this reflects the curvature of the cichlids forehead better.

Analysis of the landmarked TPS files was done in MorphoJ (version 1.05e) (Klingenberg, 2011). As this study is primarily concerned with shape differences among species I first performed a Procrustes superimposition to correct for differences in size and orientation of the fish in the picture by superimposing all landmarks of all fish in such a way that the landmark distances between the different specimens are minimal. This is done by rotating and stretching or shrinking the specimens to make them optimally fit together.

I checked for outliers using the "integrated find outliers" tool in MorphoJ. Further I corrected for allometry (often called "size correction") by performing a regression against centroid size. This step is crucial as body shape and proportion may change within a single species. Correcting for allometry ensures that observed differences between specimens are due to shape and not size. The covariance matrix resulting from the regression of coordinates against centroid size, containing superimposed and size corrected coordinates, was used in all subsequent analyses.

Of the 15 previously described haplochromine species (Snoeks, 1994; Snoeks, 2012) 9 were caught in the field campaign in spring 2012. On these 9 species I also conducted Principle Component Analysis (PCA) and Canonical Variate Analysis (CVA). I also did PCA and CVA on the full set species previously described species, and 21 additional putative new species identified from live colour photograph by Ole Seehausen. To assess the morphological distinctiveness of these groups I calculated Mahalanobis distances between all pairs of species, and their associated p-values from permutation tests (10000 permutation rounds).

To assess if the additional 21 putative species add to the morphospace already occupied by the 9 described species we calculated the area of two 95% confidence ellipses in a PC score plot. The first ellipse contains 95% of all already described specimens. The second ellipse contains 95% of all described and putative species combined. The second ellipse should occupy a larger area in the morphospace, if new species add to the morphospace.
In order to test for evidence of additional morphological diversity within the individuals that could not be assigned to putative new or existing species from live colour photos, I measured morphospace occupied by the assigned species alone and compared it to

morphospace size of all specimens, both assigned to species and unassigned. Morphospace size was estimated by calculating the area of an ellipse that includes 95% of specimens in a group of interest in the PCA plot.

Contrary to previous studies, many haplochromies were caught in the demersal zone (Paris et al, 2013). I therefore analysed shifts in morphological traits and morphospace occupation along the depth gradient. I used ANOVA to test for differences in the means among species assemblages in 5 different depth ranges.

Table 2: Species names as they are used in my thesis and their relation to names used in literature. Short additional information to each species is added.

Names used in this thesis	Names as used in Paris et al. (2013)	Names as in Snoeks (1994)	Information to species specific traits
Demersal sp1	Demersal sp1	NA	Slender body, curved forehead, crimson red caudal fin
H. graueri	*Psammochromis graueri*	*H. graueri*	Less slender, heavy head look,
H. scheffersi	*Astatotilapia scheffersi*	*H. scheffersi*	Darkish coloured body, orange caudal and anal fin
Demersal sp2	Demersal sp2	NA	Black body, crimson red caudal fin, no curvature in forehead
Demersal sp3	Demersal sp3	NA	Yellowish bright body colour, no curvature in forehead
Demersal sp4	Demersal sp4	NA	Dark green shimmering body, no curvature in forehead
Demersal sp5	Demersal sp5	NA	Dark body colour, large eyes
Demersal sp6	Demersal sp6	NA	Elongated, light coloured body
Demersal sp7	Demersal sp7	NA	Black coloured deeper body, slightly curved forehead, tip of caudal fin red
Demersal sp8	Demersal sp8	NA	Light coloured body, large eyes
Demersal sp9	Demersal sp9	NA	Bluish purple body colour, orange caudal and dorsal fin
Demersal sp10	Demersal sp10	NA	Deeper, yellow coloured body, curved forehead
H. gracilior	*Astatotilapia gracilior*	*H. gracilior*	Territorial males dark coloured, nose to beginning of dorsal fin almost straight line
H. kamiranzovu	*Yssichromis kamiranzovu*	*H. kamiranzovu*	Elongated dark body, dark fins
Littoral Black sp1	Littoral Black Lithochromis like	NA	Green, yellow colour, deep body, large head, curvature in forehead
Littoral Black sp2	Littoral Black Mbipia like	NA	Deep body, large head, curvature in forehead
Littoral Black sp3	Littoral Black Neochromis like	NA	Similar as *N. olivaceus* but brighter body colour
Littoral Black sp4	Littoral Black Pundamilia like sp1	NA	Black body colour, dark fins, large head
Littoral Black sp5	Littoral Black Pundamilia like sp2	NA	Like LB sp4 but more red in caudal, anal, dorsal fin
H. olivaceus	*Neochromis olivaceus*	*H. olivaceus*	Dark body colour, less heavy head look than other littoral sp.
Littoral light sp1	Littoral light blue and orange fin	NA	Heavy head look, deep body, bright blue colour and
Littoral light sp2	Littoral light haplochromis like	NA	Heavy head look, deep body, green, yellow or bluish body colour
Littoral light sp3	Littoral light parapabidochromis like	NA	Heavy head look, deep body, curvature in forehead, light greenish body colour
Littoral light sp4	Littoral light red dorsum	NA	Less heavy head look, greenish body colour, red dorsum
H. crebidens	*Mbipia crebidens*	*H. crebidens*	Bright blue body colour, orange caudal, anal dorsal fin, Heavy head look and deep body
H. paucidens	*Paralabidochromis paucidens*	*H. paucidens*	Less heavy head look, green, yellow body colour, orange caudal, anal dorsal fin
H. occultidens	*Lipochromis occultidens*	*H. occultidens*	Very distinct snout shape, light body colour, large eyes
Piscivorous sp1	Piscivorous sp1	NA	Similar to *H. vittatus* but more gracile, bluish body colour
Piscivorous sp2	Piscivorous sp2	NA	Body shape close to *H. gracilior,* large eyes
H. vittatus	*Pragnathochromis vittatus*	*H. vittatus*	Largest *Haplochomine sp*. in Lake Kivu, large head and mouth, predator look.

Results

In total I photographed 584 fish. Some of the photographed fish were damaged (open abdominal cavity, damaged operculum, broken tail) and some had their mouths wide open. With these individuals excluded, there were 523 specimens for further analysis.
As the first PC axis appears to reflect bending of the fish, a preservation artefact, we here provide plots of PC2 and 3 (see appendix for PC1 results).

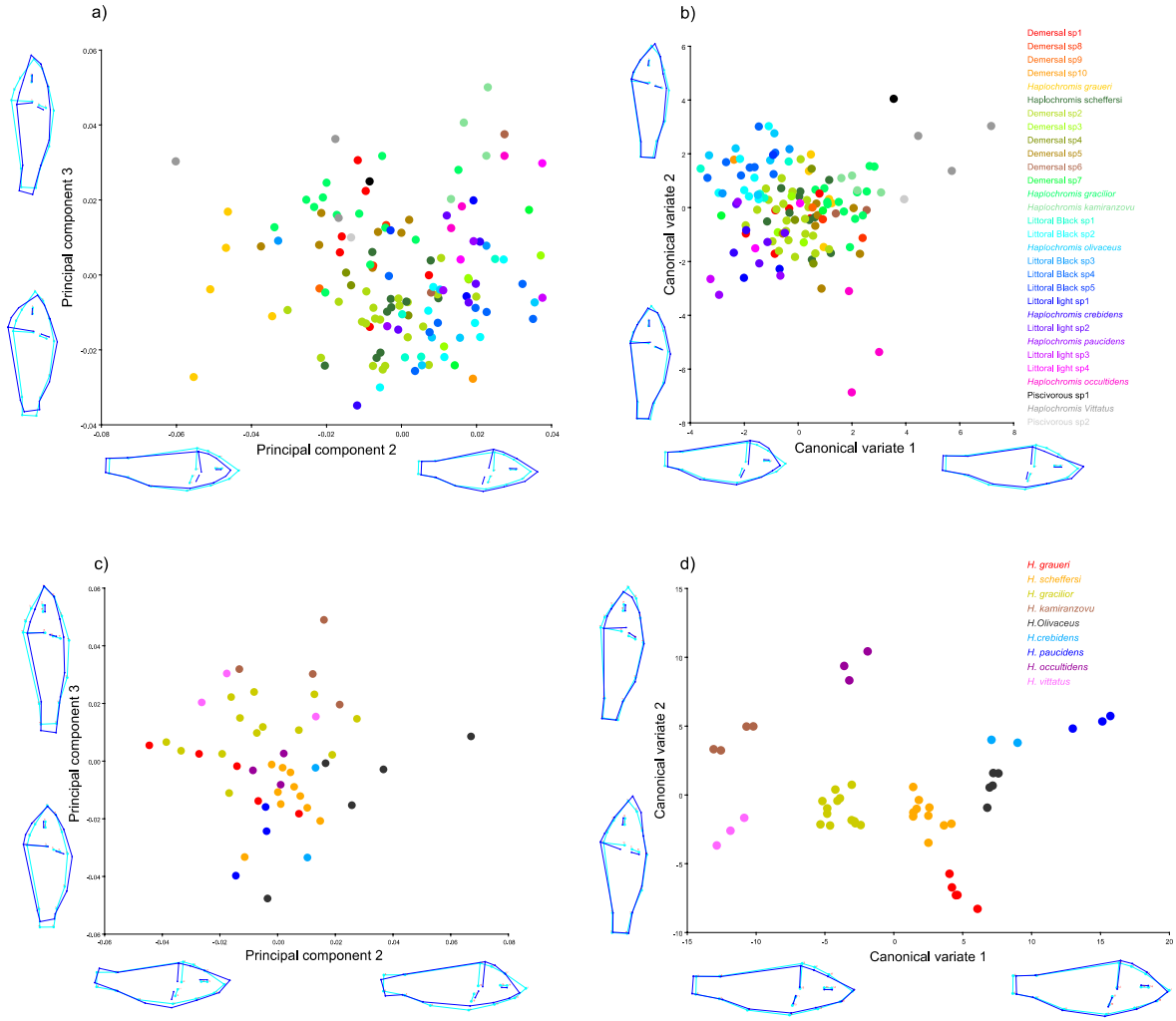

Fig. 3: PCA and CVA plots show grouping of the already described as well as of the undescribed species and gives us hints about their morphological distinctiveness. **a)** PC score plot of all assigned species. PC2 axis (18.691% of variance) shows mainly changes in head morphology whereas PC3 axis (13,814% of variance) depicts body depth. **b)** Corresponding CV score plot. Here CV1 axis shows changes in body depth (33.41% variance) and CV2 axis shows head morphology (14.42% variance). **c)** PC score plot of the 9 species in the Kivu collection that were already mentioned in Snoeks (1994). Also here PC2 axis (17, 847% of variance) shows mainly changes in head morphology although there seems to be more bending included than in the PC2 axis of Fig.4. PC3 axis (17,524% of variance) shows change in body depth. **d)** Corresponding CV score plot shows strong grouping of the 9 species and no overlap of the species in the morphospace at all. CV1 axis (56.570% of variance) shows mainly head morphology and signs of body depth whereas CV2 axis (17.927% of variance) shows head morphology and body depth again but with a stronger emphasis of body depth.

The PCA and CVA (Fig. 3) show clustering and allocation in the morphospace of all assigned species combined (Fig. 3a and Fig. 3b) and of already described species (Fig. 3c and Fig. 3d). In general, the different species are less distinguishable in analyses includ-

ing all individuals (Fig. 3a and b) than they are in the analyses of only the previously described species (Fig. 3b and c). This might be partly due to the higher number of species sharing the morphospace but also due to the species being packed together more closely in the morphospace (Fig. 3a and b). This is reflected by the mean Mahalanobis distances between the described species only 15.775, SD=4.833 (Fig. 3d) and between all specimens that got assigned to either putative new or already described species 8.0, SD=2,22 (Fig. 3b).

Figure 3b shows that most of the 30 species form a dense cloud. Within this cloud the species from the "demersal", "littoral black" and "littoral light" groups are partitioned along PC axes 2 and 3. Compared to the "demersal" species, which show tendencies to a more elongated body and shorter snout shape, the "littoral black" species have slightly deeper bodies and pointier head shapes. "Littoral light" species show similar body depth characteristics but with a tendency to shorter head morphology. Only morphologically very distinct species as *Haplochromis vittatus, "*piscivorous" sp.1 and sp.2 and *Haplochromis occultidens* are found outside of this cloud. This is also reflected in the Mahalanobis distances, as each of the Mahalanobis distance means for the three piscivorous species ("piscivorous" sp.1 = 9.603, "piscivorous" sp.2 = 10.058 and *Haplochromis vittatus* = 10.598) are higher than the overall mean Mahalanobis distance of the CVA (Fig. 3b), which is 8.0 (SD=2.22). The same is true for the mean Mahalanobis distances of *Haplochromis occultidens* (9.518). Mahalanobis distances for Fig. 3b reach a maximum of 17.477 between *Haplochromis vittatus* and "littoral light" sp.3 (p=0.254) and a minimum of 3.108 between *Haplochromis graueri* and "demersal" sp.2 (p<0.001).

The PCA in Figure 3c shows a clear clustering of each of the nine previously described species, although there is considerable overlap. In the CVA in Figure 3d however, the species form very distinct groups that are clearly separated from each other with no overlap. The Mahalanobis distances underscore this finding. The mean Mahalanobis distance is 15.775, SD=4.833 (Fig. 3d). The maximal distance is 28.656 between *Haplochromis paucidens* and *Haplochromis vittatus* (p=0.0451) and the minimal Mahalanobis distance is 8.224, found between *Haplochromis olivaceus* and *Haplochromis scheffersi* (p<0.0001).

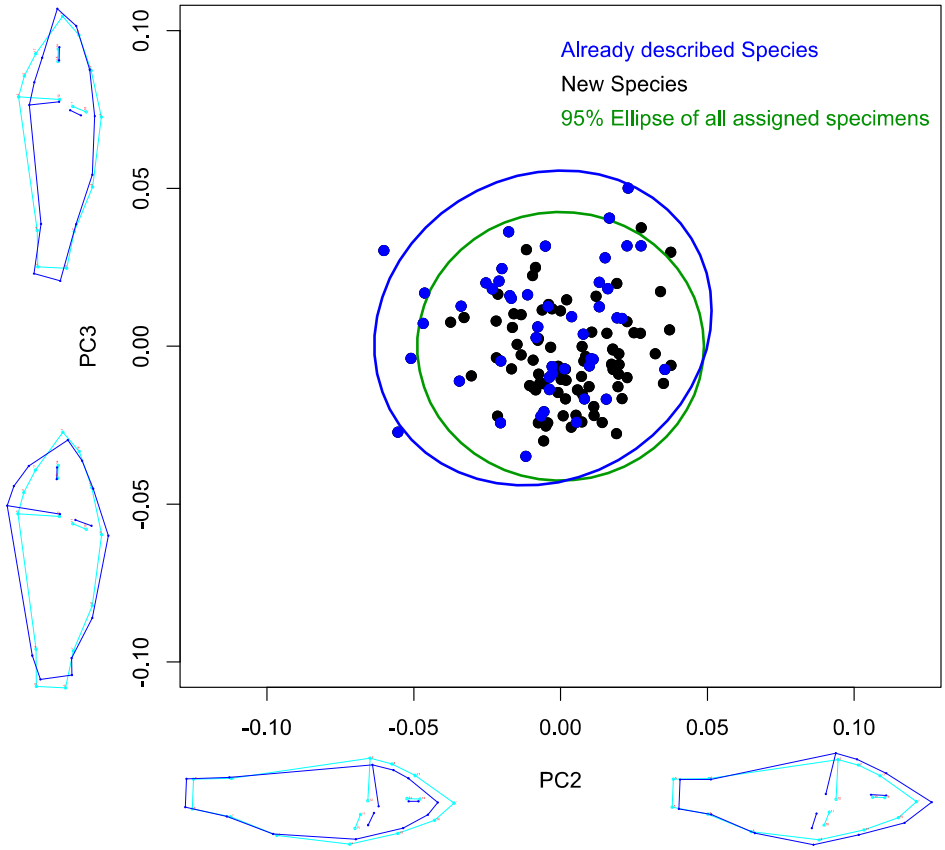

Fig. 4: PC score plot to visualize if the newly assigned species enlarge the morphospace occupied by the already assigned species. Ellipse size of already described species = 0.00895, ellipse size of all specimens = 0.00653. PC2 axis (18.691% of variance) shows mainly changes in head morphology whereas PC3 axis (13,814% of variance) depicts body depth.

Figure 4 depicts PC2 and PC3 from a PCA of all specimens combined. The black dots represent the specimens belonging to putative new species whereas the blue dots represent the specimens that belong to the already described species. Ellipse size was calculated for the already described species (0.00895) and for all specimens combined (0.00653).

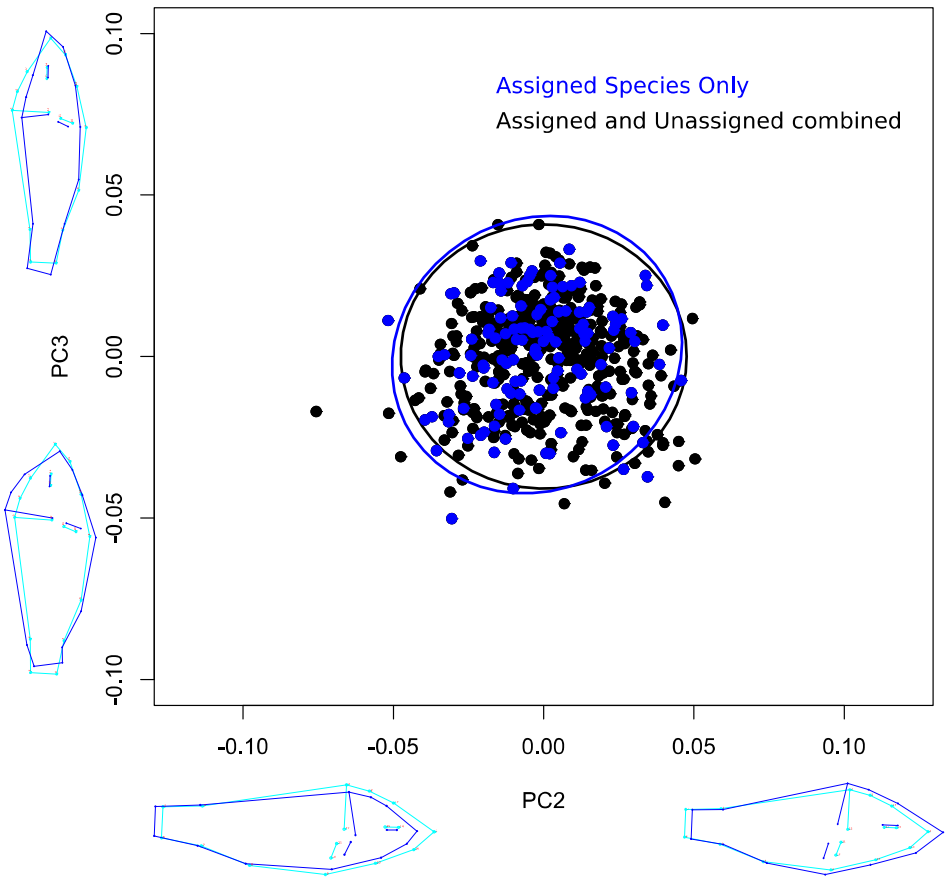

Fig. 5: PC score plot to estimate morphological diversity in unassigned specimens. Ellipse sizes do only differ very little. Ellipse size of assigned species = 0.00649, ellipse size of all specimens = 0.00612. PC2 axis explains 18,691% of variance. PC3 axis explains 13,814% of variance.

Figure 5 depicts PC2 and PC3 from a PCA of all specimens combined. Blue dots represent the specimens which were previously described or now identified by Seehausen and belonging to new putative species. Black dots represent the unidentified species. Ellipse size was calculated for the identified and described species (0.00649) and for the unidentified species (0.00612).

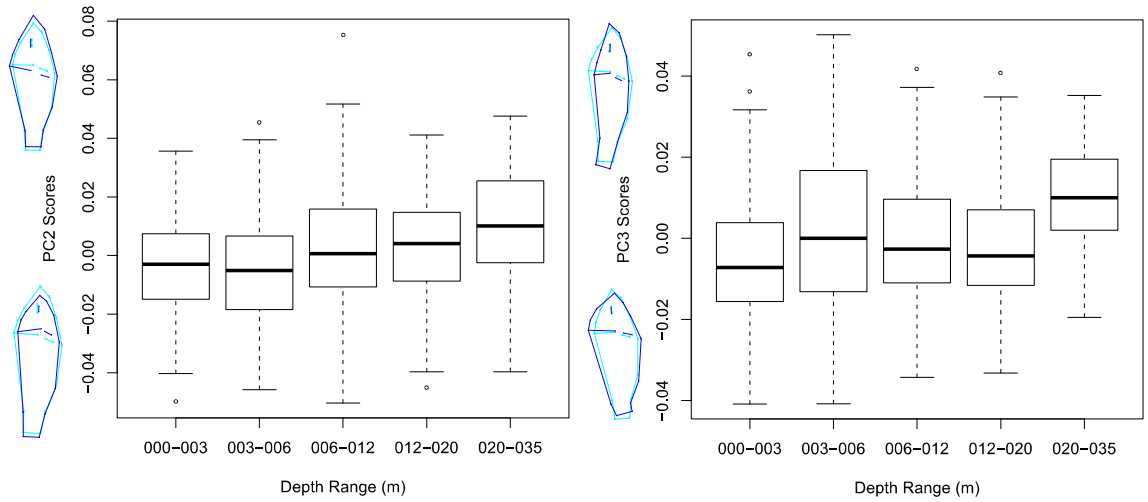

Fig. 6: Boxplots showing changes in morphology along a depth gradient. PC2 axis is head morphology. PC3 axis is body depth. PC2 axis explains 18,691% of variance. PC3 axis explains 13,814% of variance.

Depth related changes in morphology are depicted in Figure 6. These analyses shows that fish in deeper water tend to have a pointier head shape and a more elongated body. An ANOVA has been calculated for PC2 $F_{4,516}$=10.27 ($p<0.0001$) and for PC3 $F_{4,516}$=11.14 ($p<0.0001$).

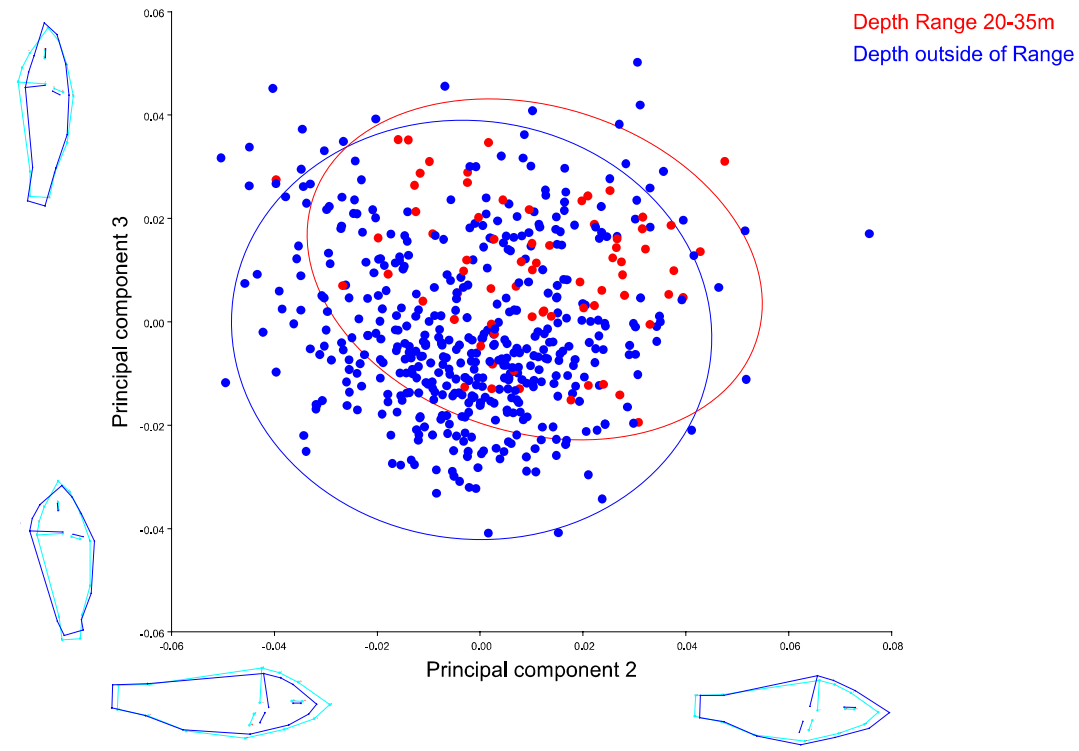

Fig. 7: The haplochromine community living below 25 meters water depth is strongly differentiated in morphology on PC axes 2 and 3. The blue dots that are here called "outside of range" are specimens caught in less than 20 meters depth. PC2 axis is head morphology. PC3 axis is body depth. PC2 axis explains 18,691% of variance. PC3 axis explains 13,814% of variance.

The finding in the PC score plot in Figure 7 is consistent with what we can see in Figure 6. The fish community between 20 and 35m has a tendency to pointier head shape and show a more elongated body.

Table 3: Species caught between 20 and 35m depth and their absolute and relative abundance in the collection.

Species caught between 20-35m depth	Absolute number of specimens caught per species	Percentage of specimens caught between 20 and 35m depth per species
H. graueri	2	33
H. scheffersi	4	36
H. gracilior	6	46
Demersal sp.2	1	5
Demersal sp.4	3	100
Demersal sp.5	7	88
Demersal sp.6	1	50
Demersal sp.8	2	100
Demersal sp.9	1	100
Littoral Black sp.3	1	50
Piscivorous sp.2	1	100

Discussion

The morphological investigation of Lake Kivu *Haplochromines* using geometric morphometric methods gives us the general impression of only a handful morphologically different and many morphologically very similar haplochromine species. This might also be a reason why Snoeks called them "notoriously difficult to identify" (Snoeks, 2012). Especially the *Haplochromis occultidens* and the piscivorous species (*Haplochromis vittatus,* "piscivorous" sp1 and sp2) have very distinct morphologies and are well separated from all the other species in morphospace (Fig. 3b). The distinctiveness of morphology might be a major criterion for the probability that a species will be taxonomically described. In this light it makes sense that *Haplochromis vittatus* was among the first fishes to be described from Lake Kivu (Boulenger, 1901). *Haplochromis occultidens,* however was described rather late (Snoeks, 1988) considering it is on the very edge of the occupied morphospace and shows a very distinct head shape. This might be due to the fact that *Haplochromis occultidens* is a rare species (Snoeks, 2012).

Aside from these morphologically very distinct exceptions, the rest of the species belonging to the "demersal", "littoral light" and "littoral black" groups form a dense cloud on PC2 and 3 (Fig. 3). Species that show considerable overlap in the morphospace might have been described from early collections due to characteristic anatomical traits such as dentition. *Haplochromis paucidens* (Regan, 1921) and *Haplochromis crebidens* (Snoeks et al., 1990) are both within the "littoral light" group and show almost total overlap in morphospace (Mahalanobis distance=7.165, $p<0.0001$), but they differ a lot in their ecology. *H. paucidens* is a typical insectivorous species and as the name suggests (lat. "paucus" = few and "dens" = tooth) has only few teeth on the oral jaws, whereas *H. crebidens* (lat. "creber"=numerous) is an epilithic algae scraper and in contrast shows numerous teeth on the oral jaws. This example shows the importance of complementing studies of overall body shape with anatomical traits of ecological importance, such as dentition, to allow a better classification of the undescribed specimens.

Morphospace ellipse size of the described species combined with the species that were identified by Seehausen is smaller (0.00653) than the ellipse size of the already described species alone (0.00895) (Figure 4). This difference in ellipse size is most likely caused by sample size differences (48 described specimens and 130 specimens described and identified in total), and this should be tested by resampling. If the ellipse sizes are not statistically significantly different, this would indicate that the 21 putative new species do not enlarge the morphospace occupied by the 9 described species that are found in our Kivu collection. This does not mean that these 21 putative new species are less likely to be real species. Rather, we have to imagine that the species with the most extreme morphologies are, as mentioned above, most likely to be described first, as they are easiest to distinguish by eye and without help of any body colouration, which fades rapidly after collection. Species with intermediate phenotypes are therefore less likely to be recognized as independent units and run the danger of being pooled with other, similar species. These results suggest that the newly identified specimens are not adding to the morphospace but are filling the morphological gaps between the already known species. This is reflected in the mean Mahalanobis distances between all 30 identified and described species (8.0, SD=2.2) and between the described species alone (15.775, SD=4.833).

The second ellipse size calculation (Fig. 5) tells us that the morphological diversity in our collection of Lake Kivu cichlids is covered to a large extent by the specimens which belong to either putative new or already described species and that morphology predicts a low chance for finding additional, morphologically different, species in the unidentified specimens. An exception to this general finding might be found in the bottom right periphery of the ellipses, where several unidentified specimens lie outside of the ellipse and virtually no identified specimens are found. It is also possible that many of the unidentified specimens are females, since they show no nuptial colouration and females of most species show a similar brown greyish colouration. Therefore, except for species with very distinct morphology, females often cannot be assigned to species (Snoeks, 2012). If female shape did not differ dramatically from conspecific males, this would be another explanation for why morphospace does not enlarge when adding the unidentified specimens.

Generally the study of haplochromine community differences in morphology along the depth gradient showed that species with deep bodies and short snouts, what is typical for algae scrapers, are restricted to shallower parts of the lake. This is the case in many "littoral black" and "littoral light" species as well as in *H. olivaceus, H. crebindes, H. paucidens* (Table A 1). Haplochromines that were caught in the deepest depth category (25-35m) in Lake Kivu have generally a more elongated body shape and pointier head and snout (Fig. 6 and Fig. 7) than species which live only in shallow waters. This is most likely explained by the assemblage of fishes that live at these greatest depths, and the ecological specialization of these fishes. Three described species and 8 putative new species were caught between 20 and 35m depth (Table 3). The three described species *H. gracilior, H. graueri* and *H. scheffersi* and the 8 identified species "demersal" sp. 2,4,5,6,8,9, "littoral black" sp. 3 and "piscivorous" sp. 2 all have in common that they have very intermediate morphologies. Variation among these species ranges between *H. gracilior* and "piscivorous" sp.2 with the pointiest snouts and "demersal" sp.2 with the shortest snout. There is almost no variation in body depth, with all 11 species caught between 20 and 35m depths having intermediate body depth. It is also interesting that

the only piscivorous species ("piscivorous" sp.2) caught at this depth is also the most intermediate looking piscivorous species by far, although here interpretations have to be treated with caution, as "piscivorous" sp.2 consists only of one single individual. Most fishes caught in the deepest depth range did also occur in shallower water. For example *Haplochromis scheffersi* and "demersal" sp2 have a wide depth distribution, ranging from 3 to 35m depth (Appendix). This makes sense, as a more elongated head shape is typical for insectivorous, piscivorous or omnivorous species, which are therefore not so much restricted to a certain depth as are algae scrapers, due to the algae's need for sunlight. Nevertheless four species in total ("demersal" sp.4, 8,9 and "piscivorous" sp.2) were only been caught between 20 and 35m depth and nowhere else. This finding points to the need for more work on the fishes that live at these depths just above Lake Kivu's oxycline, and suggests that prior taxonomic work on the fishes of this lake have not adequately sampled the diversity in the lake's deep waters.

Only a handful of studies have previously been done on the cichlid assemblage of Lake Kivu, and most of them are quite old (Boulenger, 1901; Moore, 1903; Regan 1921; Snoeks 1994). The lake's cichlid fauna is in serious need of reassessment, including more thorough sampling, thorough taxonomic assessment, and the use of genetic tools to understand the history of the group. This study provides a step towards greater understanding of the previously poorly sampled haplochromine diversity within the lake.

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Appendix

Table A 1: All described species and identified species, their depth distribution and the total number of specimens per species

Species	0-3	3-6	6-12	12-20	20-35	Specimens per species
Demersal sp1	■	■	■			**6**
H. graueri	■	■	■		■	**6**
H. scheffersi		■	■	■	■	**11**
Demersal sp2		■	■	■	■	**22**
Demersal sp3			■	■		**3**
Demersal sp4					■	**3**
Demersal sp5				■	■	**7**
Demersal sp6		■			■	**2**
Demersal sp7	■			■		**2**
Demersal sp8					■	**2**
Demersal sp9					■	**1**
Demersal sp10				■		**1**
H. gracilior	■			■	■	**13**
H. kamiranzovu	■		■	■		**4**
Littoral Black sp1	■	■				**4**
Littoral Black sp2	■	■	■			**7**
Littoral Black sp3		■			■	**2**
Littoral Black sp4	■	■				**8**
Littoral Black sp5			■			**1**
H. olivaceus	■	■	■			**5**
Littoral light sp1	■					**2**
Littoral light sp2	■					**5**
Littoral light sp3	■					**1**
Littoral light sp4	■	■				**2**
H. crebidens	■	■				**2**
H. paucidens	■	■		■		**3**
H. occultidens	■	■				**3**
Piscivorous sp1			■			**1**
Piscivorous sp2					■	**1**
H. vittatus	■	■		■		**4**

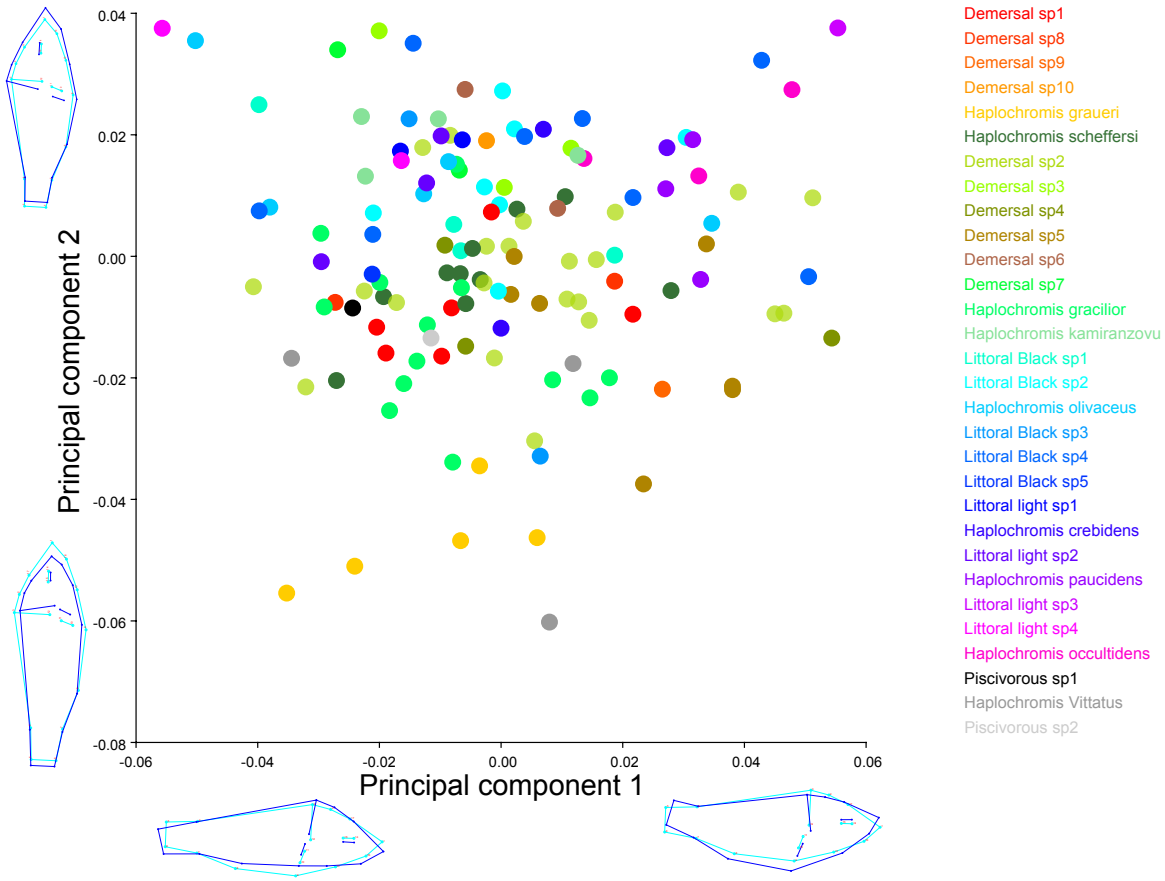

Fig A 1: PCA plot of all identified as well as of all described specimens. PC1 axis (26.84% of variance) shows mainly bending of the fish, a preservation artefact. PC2 axis (18.691 % of variance) depicts head morphology.

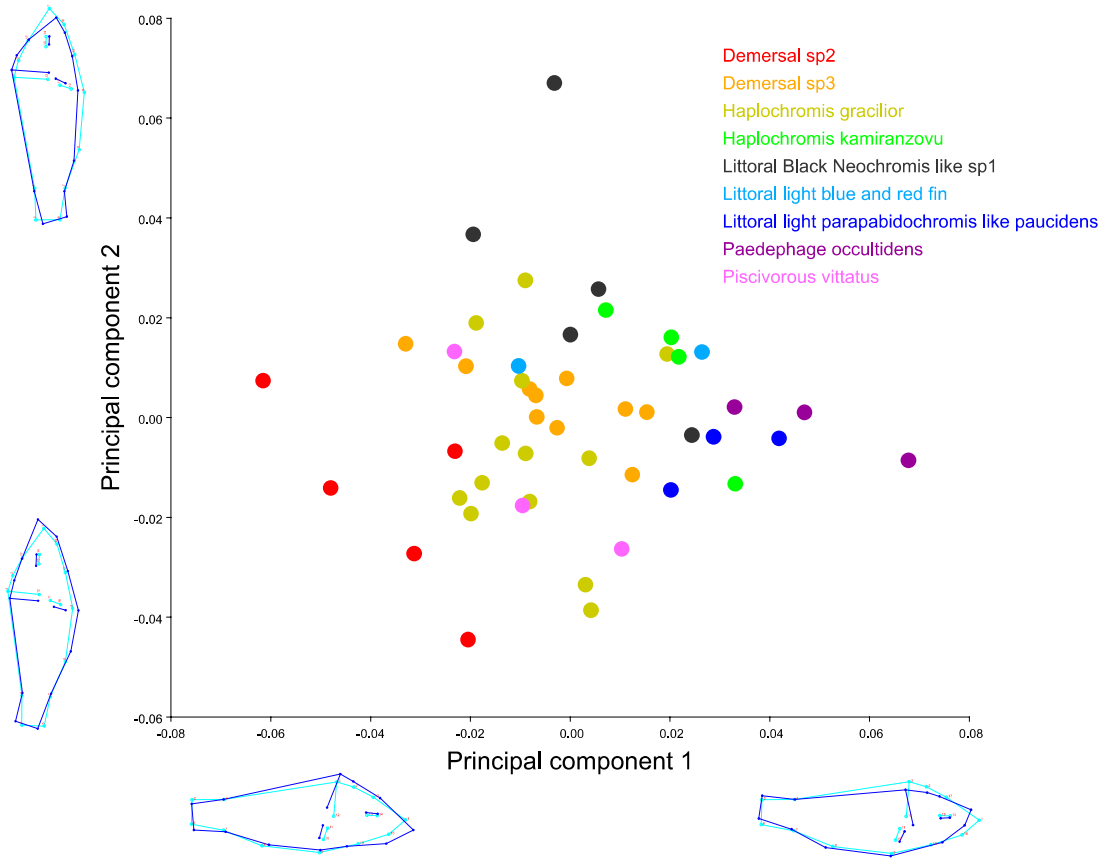

Fig A 2: PCA plot of all described species. PC1 axis (27.33% of variance) shows mainly bending of the fish, a preservation artefact. PC2 axis (17.85 % of variance) depicts head morphology and again slight bending of the fish.

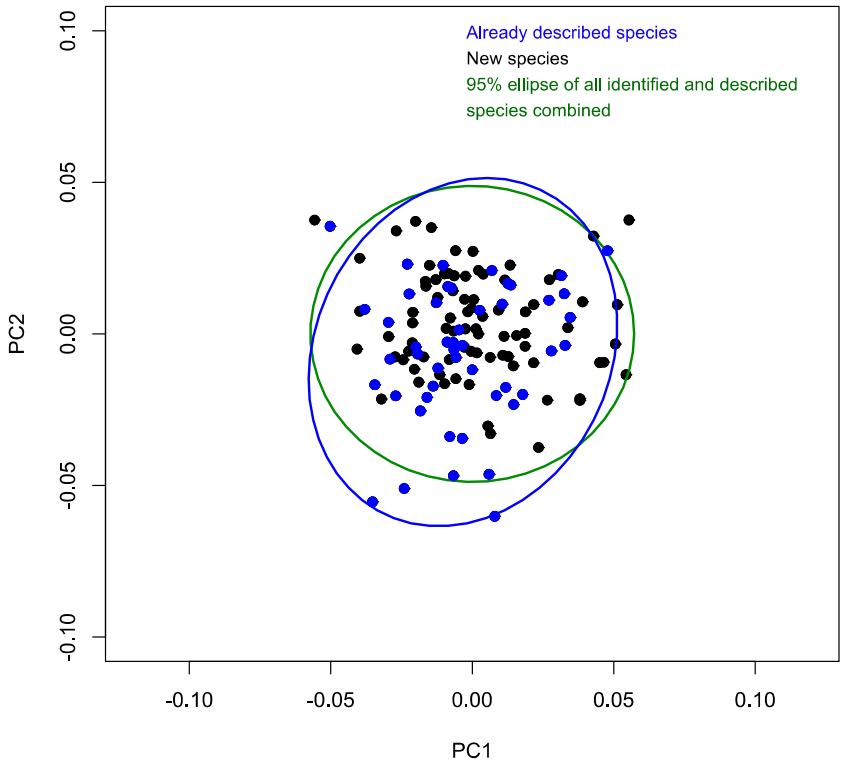

Fig A 3: PC score plot to visualize if the newly assigned species enlarge the morphospace occupied by the already assigned species. Ellipse size of already described species = 0.00971, ellipse size of all specimens = 0.00877. PC1 axis (26,619% of variance) shows mainly bending of the fish (see Fig. A 1) whereas PC2 axis (19.391% of variance) depicts Head morphology.

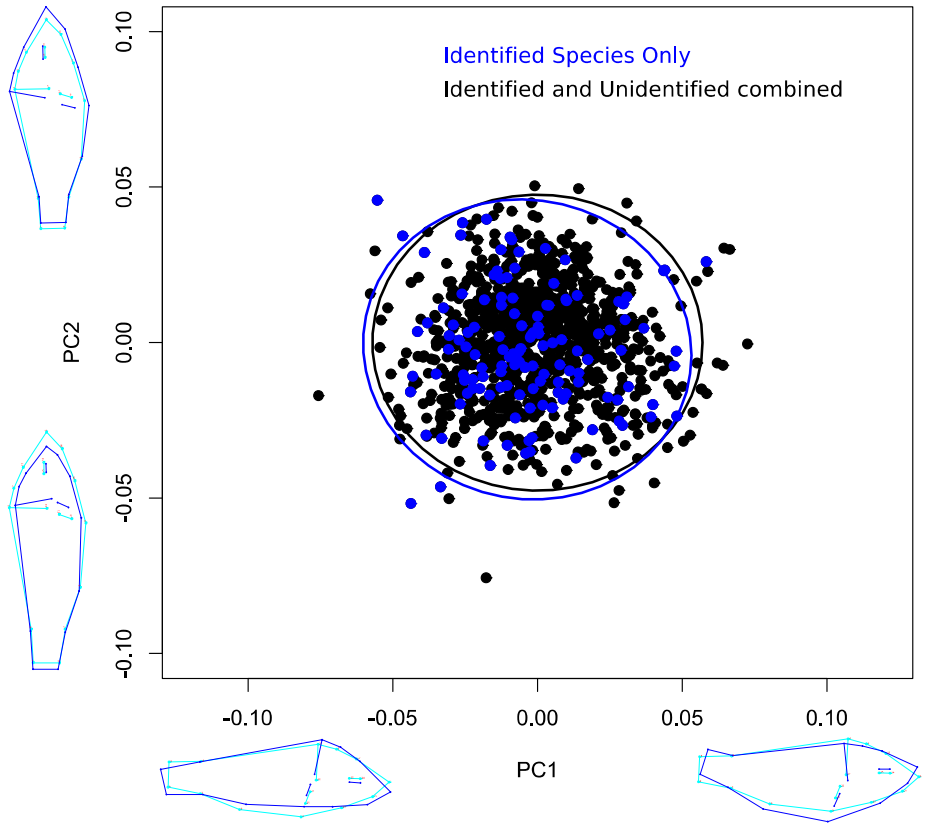

Fig A 4: PC score plot to estimate morphological diversity in unassigned specimens. Ellipse size of assigned species = 0.00859, ellipse size of all specimens = 0.00852. PC1 axis explains 26.836% of variance. PC2 axis explains 18.691% of variance.

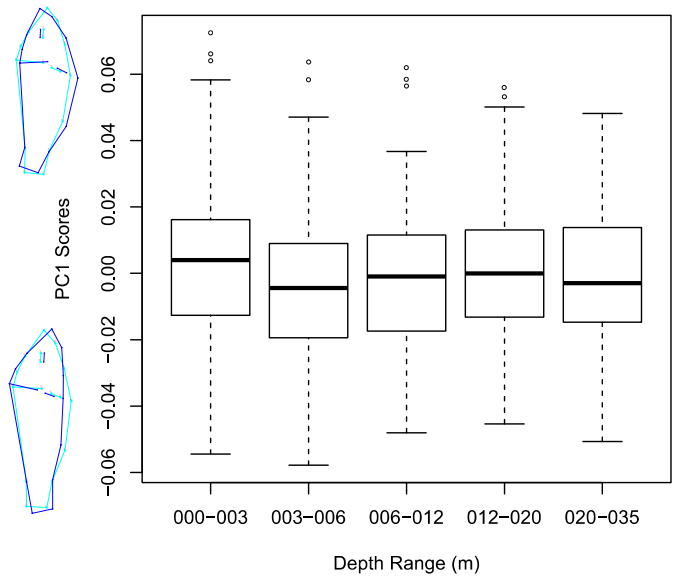

Fig A 5: Boxplots showing changes in morphology along a depth gradient. PC1 axis is bending of the fish, a preservation artefact. PC1 axis explains 26.836% of variance.

To compare the variation of the means in the five groups an ANOVA has been calculated $F_{4,516}$=1,836, p=0.121.

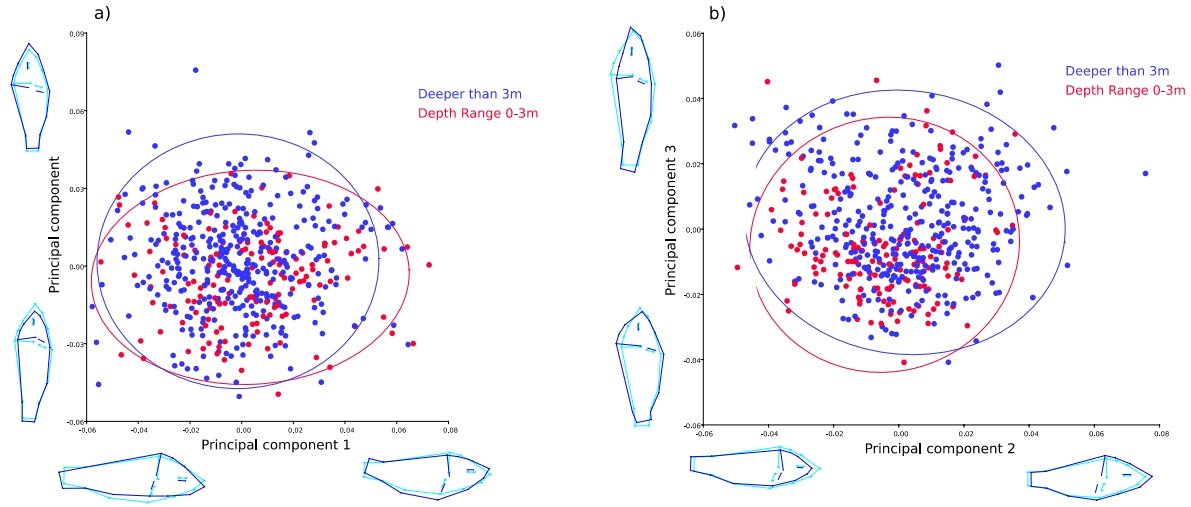

Fig A 6: Morphospace occupation from haplochromines living in the shallowest depth range from 0-3m depth compared to all deeper living specimens. **a)** PC1 versus PC2 score plot. PC1 axis depicts bending of fish body and explains 26.836% of variance. PC2 axis depicts head morphology and explains 18.691% of variance. **b)** PC2 versus PC3 score plot. PC3 depicts body depth and explains 13.814% of variance.

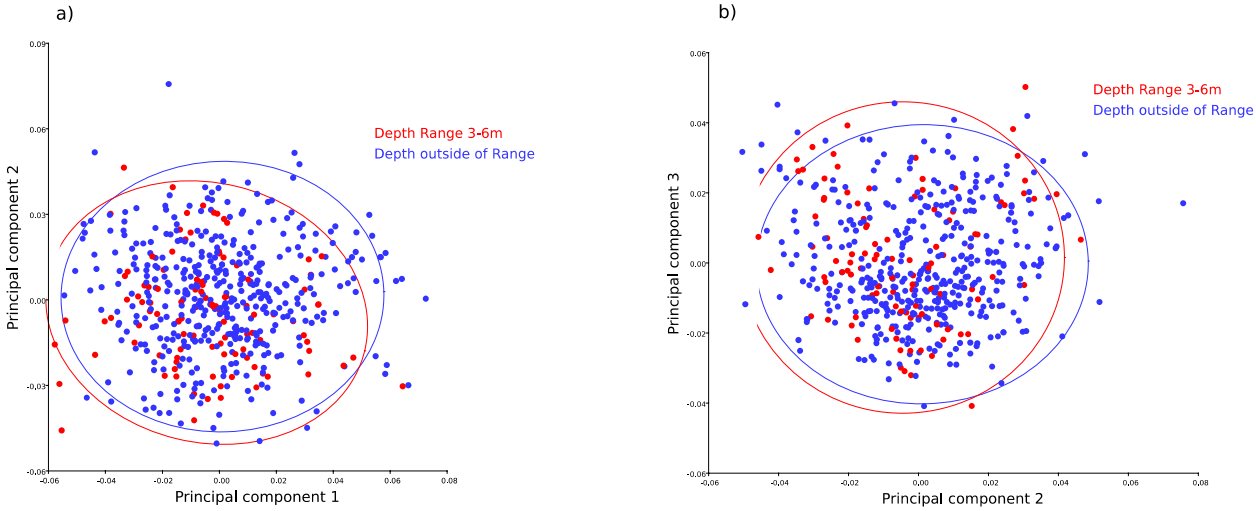

Fig A 7: Morphospace occupation from haplochromines living in the depth range from 3-6m depth compared to all specimens living in other depth ranges. **a)** PC1 versus PC2 score plot. PC1 axis depicts bending of fish body and explains 26.836% of variance. PC2 axis depicts head morphology and explains 18.691% of variance. **b)** PC2 versus PC3 score plot. PC3 depicts body depth and explains 13.814% of variance.

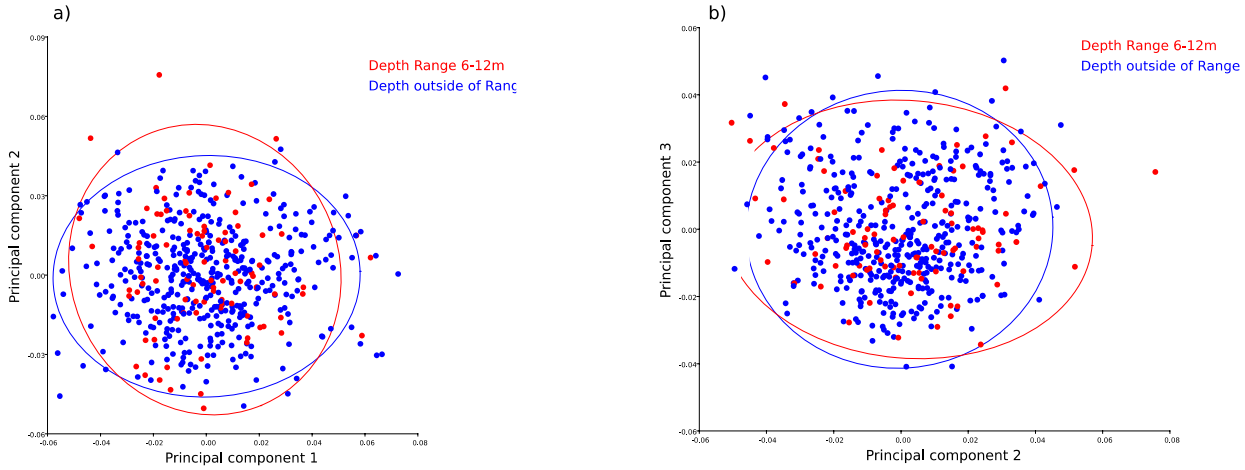

Fig A 8: Morphospace occupation from haplochromines living in the depth range from 6-12m depth compared to all specimens living in other depth ranges. **a)** PC1 versus PC2 score plot. PC1 axis depicts bending of fish body and explains 26.836% of variance. PC2 axis depicts head morphology and explains 18.691% of variance. **b)** PC2 versus PC3 score plot. PC3 depicts body depth and explains 13.814% of variance.

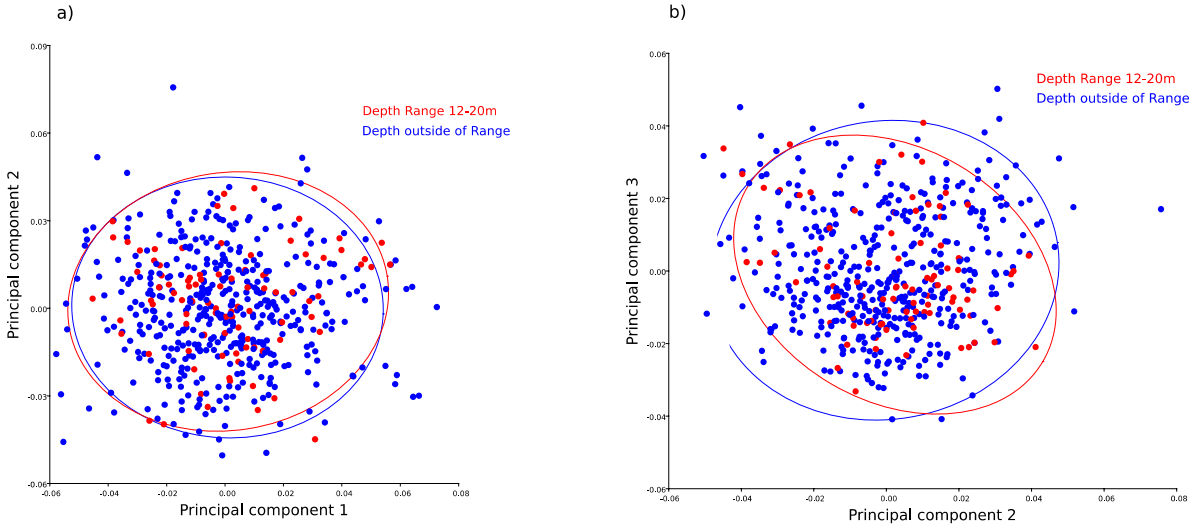

Fig A 9: Morphospace occupation from haplochromines living in the depth range from 12-20m depth compared to all specimens living in other depth ranges. **a)** PC1 versus PC2 score plot. PC1 axis depicts bending of fish body and explains 26.836% of variance. PC2 axis depicts head morphology and explains 18.691% of variance. **b)** PC2 versus PC3 score plot. PC3 depicts body depth and explains 13.814% of variance.

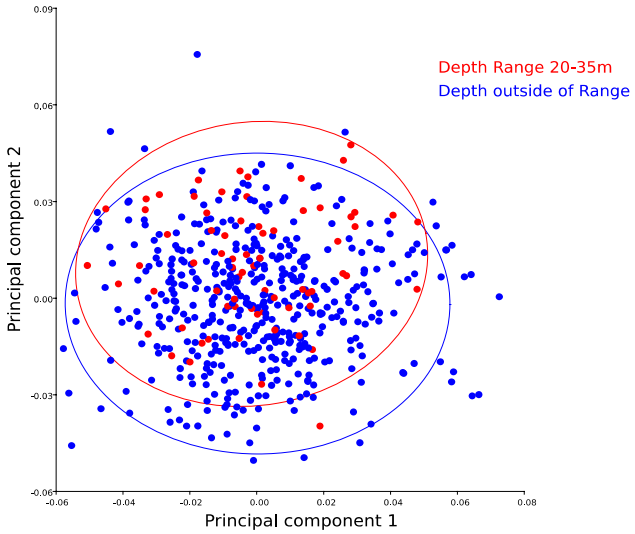

Fig A 10: Morphospace occupation from haplochromines living in the depth range from 20-35m depth compared to all specimens living in other depth ranges. PC1 axis depicts bending of fish body and explains 26.836% of variance. PC2 axis depicts head morphology and explains 18.691% of variance

Table A 2: Mahalanobis distances among groups and p-values from permutation tests (10000 permutation rounds) extracted from a CVA which considers only previously described species. All p-values in red and bolt are <0.05. Bright yellow background = highest Mahalanobis distance, orange background = lowest Mahalanobis distance.

	1	2	3	4	5	6	7	8	9
1. H. crebidens	0	14.4098	14.1371	21.6442	16.1828	11.4464	11.9827	13.4833	23.5335
2. H. gracilior	**0.0014**	0	11.0506	11.0229	12.2858	14.0171	20.2108	10.1193	10.7312
3. H. graueri	**0.0444**	**0.0001**	0	20.5083	18.9748	11.8854	16.4424	10.4861	18.628
4. H. kamiranzovu	**0.0282**	**0.0002**	**0.0031**	0	13.4267	19.9369	27.0794	16.7702	9.7683
5. H. occultidens	0.1051	**0.0014**	**0.0008**	**0.0171**	0	16.0242	19.6759	13.4379	16.1772
6. H. olivaceus	**0.007**	**0.0003**	**0.0031**	**0.0049**	**0.0085**	0	13.4861	8.2235	20.1782
7. H. paucidens	**0.0001**	**0.0002**	**0.0055**	**0.009**	0.0958	**0.0012**	0	16.9473	28.6561
8. H. scheffersi	**0.002**	**0.0001**	**0.0001**	**0.0003**	**0.0012**	**0.0001**	**0.0006**	0	14.9315
9. H. vittatus	**0.0318**	**0.0016**	**0.0084**	**0.0006**	**0.0001**	**0.0122**	**0.0451**	**0.0031**	0

Table A 3: Mahalanobis distances among groups and p-values from permutation tests (10000 permutation rounds) extracted from a CVA which considers all putative new species as well as the 9 previously described species. All p-values in red and bolt are <0.05. Bright yellow background = highest Mahalanobis distance, orange background = lowest Mahalanobis distance.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1. Demersal sp1	0	0.0346	0.5593	0.1401	0.0016	0.0001	0.0001	0.0125	0.0096	0.0005	0.0332	0.0325	0.0001	0.0044
2	2. Demersal sp8	7.7077	0	0.3379	0.3321	0.0068	0.001	0.0029	0.036	0.0668	0.2976	0.3336	0.3328	0.1472	0.0514
3	3. Demersal sp9	7.096	9.7397	0	1	0.0765	0.0185	0.0754	0.0795	0.1626	0.0513	0.0001	0.3375	0.0546	0.1968
4	4. Demersal sp10	8.2477	9.2564	9.2902	0	0.0116	0.0797	0.1291	0.498	0.1654	0.0346	0.0001	0.3332	0.0625	0.1941
5	5. *H. graueri*	8.1292	7.2753	9.9633	11.1847	0	0.0001	0.0001	0.004	0.0092	0.0006	0.0044	0.0348	0.0001	0.0035
6	6. *H. scheffersi*	6.2505	5.3862	7.5436	6.8271	7.901	0	0.0001	0.0029	0.0993	0.0001	0.0222	0.0073	0.0001	0.0011
7	7. Demersal sp2	5.275	6.441	7.1785	6.651	7.9706	3.1088	0	0.0002	0.0002	0.0001	0.0281	0.112	0.0001	0.0002
8	8. Demersal sp3	8.5641	8.622	7.8723	7.2926	11.2334	5.6344	5.4333	0	0.066	0.0031	0.0673	0.5671	0.001	0.0186
9	9. Demersal sp4	7.3029	5.967	8.0334	8.0366	8.0458	4.001	5.1798	7.051	0	0.3884	0.1008	0.0673	0.0027	0.0302
10	10. Demersal sp5	6.5882	4.389	8.8034	9.0609	7.2551	4.5743	5.104	8.1754	3.7132	0	0.0129	0.022	0.0002	0.0025
11	11. Demersal sp6	7.557	7.9418	8.5844	9.2274	9.7946	5.3491	5.2046	5.7794	6.956	6.8191	0	0.0001	0.0073	0.0458
12	12. Demersal sp7	7.4472	8.0158	8.6082	7.8079	9.6214	5.2931	4.5479	4.9636	7.0534	7.243	6.2375	0	0.0084	0.0301
13	*13. H. gracilior*	5.611	4.7491	7.9222	9.0786	6.3217	4.4335	5.0169	7.5725	4.6206	3.6709	5.5648	6.613	0	0.0001
14	*14. H. kamiranzovu*	8.1406	7.799	9.4478	9.9458	10.6678	6.4267	6.9531	7.3132	6.1592	6.3724	5.9035	7.0941	5.7948	0
15		5.2503	7.9065	8.0443	6.4707	9.2989	5.1168	4.1338	7.0192	6.4502	6.6183	6.9659	5.9366	6.3666	7.5619
16		5.8992	8.7088	8.151	5.6157	9.8235	5.3932	4.1087	6.1343	6.9228	7.3795	6.9682	4.912	7.0346	8.0007
17		6.3906	8.0069	8.2356	5.4888	9.1095	4.633	4.0494	5.9183	6.737	7.228	6.0425	4.8798	6.6055	7.4934
18		6.3892	7.0716	8.0797	7.3607	7.9892	5.4337	6.0026	7.5712	6.2155	6.2391	7.9591	6.5833	5.6967	7.4283
19		6.8524	9.4962	8.6699	6.7139	10.3818	5.713	4.3329	5.9746	7.4118	7.9297	6.1205	4.665	7.3542	7.6002
20		8.7015	8.766	9.9153	8.3669	9.8722	7.4806	7.2009	7.5772	7.7473	7.9875	9.112	7.9732	7.9324	8.7229
21		6.3847	8.8461	9.2088	8.9987	10.7178	6.6073	5.5412	6.9879	7.0413	7.3626	7.7901	6.4672	7.3623	7.7078
22		5.6141	8.2808	8.0434	6.6687	9.7092	5.5141	4.283	6.2501	7.1533	7.0868	7.2055	5.7178	6.8543	8.7826
23		5.2651	7.6217	6.9758	6.4568	9.5904	4.4394	4.2017	5.5187	5.2689	6.1294	6.2706	5.5706	5.9261	6.625
24		7.2992	10.3916	7.7287	9.1959	10.8591	6.7394	6.061	7.0823	7.6314	8.3411	7.653	7.3157	8.5961	8.6988
25		11.5083	14.268	10.5409	11.2914	15.423	11.0338	10.2272	9.8206	11.8868	12.7918	11.1068	10.0052	12.9257	12.6566
26		8.4129	9.0071	10.9924	9.7349	11.5118	7.3083	7.2088	7.7468	7.1533	6.8295	7.7909	6.9464	7.0899	6.755
27		9.392	9.3258	11.268	11.981	12.7156	8.2299	7.8441	8.8468	8.92	7.8915	7.4972	8.7155	8.7365	8.4619
28		10.7404	9.9164	10.4974	11.7762	11.4019	7.947	8.8935	9.0448	8.3248	8.6198	7.6818	10.219	8.3009	8.2677
29		10.247	7.4565	11.2884	12.4818	8.6065	9.0659	9.5817	11.736	8.8609	7.502	8.9902	11.2423	6.5077	8.8548
30		9.8804	8.0591	11.8165	11.1555	8.1708	8.1435	8.6667	10.5189	8.303	7.59	8.1818	9.6518	6.5468	7.7893

15. Littoral Black sp1	16. Littoral Black sp2	17. *H. olivaceus*	18. Littoral Black sp3	19. Littoral Black sp4	20. Littoral Black sp5	21. Littoral light sp1	22. *H. crebidens*	23. Littoral light sp2	24. *H. paucidens*	25. Littoral light sp3	26. Littoral light sp4	27. *H. occultidens*	28. Piscivorous sp2	29. *H. vittatus*	30. Piscivorous sp1
9.3191	9.6433	8.7757	9.2464	9.8569	8.4351	11.5701	11.2058	10.2123	11.8565	16.7448	9.8755	12.0523	9.6116	5.6173	0
10.5799	11.4452	10.8646	10.1401	11.8921	10.469	12.6904	11.6431	11.362	13.3132	17.477	11.0565	11.6769	10.6891	0	0.501
10.065	10.5897	9.9084	10.608	10.2431	11.2487	10.5278	11.2659	8.8765	9.8097	13.2062	9.8853	10.1124	0	0.171	1
9.0983	9.1205	10.1395	11.3071	9.5622	11.2588	8.5335	9.0949	8.4692	8.5742	11.2216	9.0048	0	0.249	0.0852	0.0841
6.7151	7.8812	7.8159	7.4187	7.793	8.9035	6.9844	7.2938	6.6284	8.8953	12.1543	0	0.0334	0.3236	0.0656	0.3309
9.9975	9.4898	10.6268	12.1824	9.2349	13.6343	10.6702	9.8195	9.2704	8.5485	0	0.3379	0.2497	1	0.2539	1
6.3196	6.1516	6.7845	8.5719	6.6004	9.2562	6.5829	7.1645	5.9219	0	0.2562	0.1057	0.0919	0.1655	0.1005	0.1643
4.7107	4.948	4.7234	5.947	5.2401	7.9983	5.3917	4.6706	0	0.0404	0.182	0.0123	0.0001	0.0001	0.0089	0.0309
4.9398	4.8971	5.5676	6.1064	5.3323	8.3118	6.1769	0	0.3331	0.0001	0.0001	0.3268	0.0302	0.3294	0.0666	0.3348
6.6391	6.484	7.4638	8.1699	7.1707	9.1458	0	0.3396	0.1292	0.206	0.3329	0.0001	0.0001	0.3338	0.0638	0.0001
7.7275	6.8361	6.9975	7.7354	8.0343	0	0.3335	0.3403	0.0166	0.0001	1	0.3336	0.1627	1	0.0824	1
4.6555	3.4476	3.614	6.6428	0	0.1616	0.0083	0.1593	0.0001	0.0011	0.005	0.0023	0.0006	0.0294	0.002	0.1092
6.3343	6.9541	5.7888	0	0.0071	0.6658	0.3382	0.0001	0.0111	0.0001	0.0001	0.3356	0.0001	0.3359	0.1026	0.3287
4.1908	3.8408	0	0.0922	0.1919	0.1438	0.0145	0.0966	0.0317	0.0033	0.1016	0.0479	0.002	0.0453	0.0119	0.1406
3.8125	0	0.0563	0.0003	0.066	0.1887	0.0055	0.0886	0.0002	0.0008	0.0686	0.0094	0.0048	0.0497	0.0025	0.0164
0	0.1188	0.1347	0.0333	0.0175	0.1349	0.0559	0.3309	0.0408	0.0287	0.1874	0.0716	0.026	0.1817	0.0274	0.1583
0.0284	0.0002	0.0066	0.0105	0.0013	0.018	0.0628	0.0622	0.0095	0.0242	0.2007	0.0515	0.0246	0.1715	0.0296	0.0509
0.0002	0.0001	0.0001	0.0021	0.0001	0.0336	0.0008	0.0032	0.0001	0.001	0.0648	0.0069	0.0018	0.0654	0.0026	0.2314
0.1344	0.1922	0.19	0.3242	0.4132	0.3315	0.3244	0.3351	0.005	0.0352	0.0001	0.338	0.0351	0.3383	0.067	0.3276
0.0638	0.0019	0.0047	0.3383	0.0297	0.0001	0.0001	0.331	0.0054	0.0674	0.3367	0.3304	0.0001	0.3328	0.0968	0.3285
0.0003	0.0001	0.0001	0.0048	0.0001	0.0248	0.0224	0.0006	0.0001	0.0078	0.0733	0.0137	0.0012	0.1003	0.006	0.0818
0.0284	0.0015	0.0055	0.0645	0.0032	0.1648	0.0299	0.0347	0.0037	0.0211	0.2446	0.0657	0.0321	0.1658	0.1041	0.1678
0.0555	0.0041	0.0133	0.066	0.0022	0.1687	0.0665	0.0661	0.0014	0.0538	0.0806	0.0688	0.0346	0.1604	0.0542	0.1678
0.0007	0.0001	0.0001	0.0034	0.0001	0.0613	0.0066	0.2454	0.0004	0.0005	0.0184	0.0038	0.0001	0.036	0.0004	0.0244
0.0001	0.0001	0.0001	0.0144	0.0001	0.0824	0.0037	0.0089	0.0002	0.0027	0.0268	0.0165	0.0005	0.0408	0.0038	0.0798
0.0035	0.0001	0.0001	0.0054	0.0003	0.007	0.0513	0.0214	0.0036	0.0119	0.0335	0.0077	0.0177	0.0299	0.0023	0.0054
0.1852	0.7254	0.828	0.3369	0.1083	1	0.3331	0.6635	0.2082	0.0001	0.0001	0.3352	0.1653	1	0.2502	1
0.1985	0.0344	0.0328	0.3265	0.1137	1	0.327	0.3385	0.0992	0.1644	1	0.0001	0.2523	1	0.2471	1
0.0608	0.0095	0.0014	0.3349	0.0047	0.3331	0.33	0.339	0.0189	0.0984	0.3348	0.3305	0.0986	0.336	0.0988	0.3315
0.0244	0.0005	0.0035	0.0276	0.0003	0.1321	0.0676	0.1835	0.015	0.0112	0.1352	0.0284	0.0039	0.1324	0.0133	0.115

E r k l ä r u n g

gemäss Art. 28 Abs. 2 RSL 05

Name/Vorname: .....
Matrikelnummer: .....
Studiengang: ……

Bachelor ☐ Master ☐ Dissertation ☐

Titel der Arbeit: .....
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