

## **SM. Details on the different MWU tests performed between the six Luansa populations**

### **For the meristics**

**SM1.** MWU tests meristic variation in female populations: The MWU tests were first performed between the females of the six Luansa populations, separately and this for three of the four meristic characters included in the first PCA as the number of anal fin rays revealed to be invariable between populations (Table A6). These tests showed no significant differences between all five upstream Luansa populations compared to each other, but each showed significant differences to the population from downstream the last Sanshifolo Falls. Therefore, the five upstream populations, together, and the single downstream population of the Luansa were tentatively considered as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

**SM2.** MWU tests meristic variation in male populations: The MWU tests were first performed between the males of the six Luansa populations, separately and this for three of the four meristic characters included in the second PCA as the number of anal fin rays revealed to be invariable between populations (Table A7). These tests showed no, except for one, significant differences between all five upstream Luansa populations compared to each other, but each showed significant differences to the population from downstream the last Sanshifolo Falls. Therefore, the five upstream populations, together, and the single downstream population of the Luansa were tentatively considered each as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

**SM3.** MWU tests meristic variation between both sexes and for all populations: The MWU tests were first performed between the males and females of the sexed Luansa populations (Table A9b). These tests showed that only one, i.e., the total number of pectoral fin rays, out of the four meristic characters tested, was significantly different ( $P \leq 0.05$ ) between males and females of the IntP2 population only (Table A9b). Nevertheless, considering that, for the females and the males of the five upstream populations, respectively, no or only one significant difference was found, whereas significant differences were found between each of them and the most downstream population (see above SM1 and SM2), these five upstream populations, together, on the one hand, and the single downstream population of the Luansa, on the other hand, were tentatively considered each as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

## For the measurements

**SM4.** MWU tests variation in measurements in female populations: The MWU tests were first performed between the females of the six Luansa populations separately and this for all measurements taken on the females (Table A10). However, these were not performed for one comparison, i.e., between the females of *K. sp.* 'lua-upstream'\_IntP1 vs. *K. sp.* 'lua-upstream'\_IntP3, due to significant size class (standard length) differences (Table A15). These tests showed no significant differences between all five upstream populations compared to each other, but each showed significant differences to the population from downstream the last Sanshifolo Falls. Therefore, the five upstream populations and the single downstream population of the Luansa were tentatively considered each as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

**SM5.** MWU tests variation in measurements in male populations: The MWU tests were first performed between the males of the six Luansa populations separately and this for all measurements taken on the males (Table A12b). However, these were not performed between *K.* 'lua-upstream'\_KP2 vs. *K.* 'lua-upstream'\_Int1, *K.* 'lua-upstream'\_KP2 vs. *K.* 'lua-upstream'\_Int3, *K.* 'lua-upstream'\_Int1 vs. *K.* 'lua-upstream'\_Int2, *K.* 'lua-upstream'\_Int1 vs. *K.* 'lua-upstream'\_Int3 and *K.* 'lua-upstream'\_Int2 vs. *K.* 'lua-upstream'\_Int3 due to significant different size class (standard length) differences (Table A15). These tests showed no significant differences between all five upstream populations compared to each other, except pre-anal distance which was significantly different between *K.* 'lua-upstream'\_KP1 vs. *K.* 'lua-upstream'\_Int2. But, instead, each of these populations shows significant differences to the population from downstream the last Sanshifolo Falls. Therefore, the five upstream populations and the single downstream population of the Luansa were tentatively considered each as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

**SM6.** MWU tests variation in measurements in between both sexes and for all populations: The MWU tests were first performed between the males and females of the six Luansa populations separately (Table A14b). Three to six significant differences, of which zero to four highly significant, were found between the males and females of each of the five upstream populations of the Luansa River (Table A14b). As the five most upstream populations of the Luansa show no significant differences between each other, but each shows significant differences to the most downstream populations from below the Sanshifolo Falls these are put together to compare them with the other populations/species. Nevertheless, considering that, for the females and the males of the five upstream populations, respectively, no or only one significant difference was found, whereas significant differences were found between each of them and the most downstream population (see above SM4 and SM5), these five upstream populations, on the one hand, and the single downstream population of the Luansa, on the other hand, were tentatively considered each as two distinct taxonomic units, which were thus compared with each other and the two other, most similar, species.

**Table S1.** Type region and overall distribution according to both, ichthyofaunal provinces (IPs) and ecoregions (ECRs) for the 13 valid *Kneria* species, in alphabetic order. Ichthyofaunal Provinces (IPs): A: Angolan; Ca: Cape; Co: Congolese; Ea: Eastern; GLEA: Great Lakes of East Africa; and Z: Zambezi (sensu Levêque & Paugy [64]). Ecoregions (ECRs): BM: Bangweulu–Mweru; ECB: Eastern Coastal Basin; KWA: Kwanza; KAS: Kasai; LTA: Lake Tanganyika; LR: Lake Rukwa; PAN: Pangani; STH: South Temperature Highveld; UC: upper Congo; UL: upper Lualaba; UZF: upper Zambezi Floodplains; ZH: Zambezian Headwaters; and ZLV: Zambezian Lowveld (sensu Thieme et al. [68]). Data presented mainly based on Poll [2,20], Fricke et al. [8], Froese & Pauly [22] and RMCA collection holdings. +: present; -: absent; <sup>T</sup> = type region; and ? = doubtful presence.

| Species                                      | IPs  | A              | Ca  | Co             |     |                |                | Ea             |                |                | GLEA | Z              |                |     |
|--|------|----------------|-----|----------------|-----|----------------|----------------|----------------|----------------|----------------|------|----------------|----------------|-----|
|  | ECRs | KWA            | STH | BM             | KAS | UC             | UL             | LR             | ECB            | PAN            | LTA  | UZF            | ZH             | ZLV |
| <i>K. angolensis</i> Steindachner, 1866      |      | + <sup>T</sup> | –   | –              | +?  | –              | –              | –              | –              | –              | –    | +              | +              | –   |
| <i>K. ansorgii</i> (Boulenger, 1910)         |      | + <sup>T</sup> | –   | –              | +?  | +?             | +?             | –              | –              | –              | –    | –              | –              | –   |
| <i>K. auriculata</i> Pellegrin, 1905         |      | –              | +   | +?             | –   | +?             | +?             | –              | –              | –              | –    | +              | + <sup>T</sup> | +   |
| <i>K. katangae</i> Poll, 1976                |      | –              | –   | –              | –   | –              | + <sup>T</sup> | –              | –              | –              | –    | –              | –              | –   |
| <i>K. maydelli</i> Ladiges & Voelker, 1961   |      | –              | –   | –              | –   | –              | –              | –              | –              | –              | –    | –              | + <sup>T</sup> | –   |
| <i>K. paucisquamata</i> Poll & Stewart, 1975 |      | –              | –   | + <sup>T</sup> | –   | –              | –              | –              | –              | –              | –    | –              | –              | –   |
| <i>K. polli</i> Trewavas, 1937               |      | –              | –   | –              | +?  | –              | –              | –              | –              | –              | –    | + <sup>T</sup> | +              | +   |
| <i>K. ruaha</i> Seegers, 1995                |      | –              | –   | –              | –   | –              | –              | –              | + <sup>T</sup> | –              | –    | –              | –              | –   |
| <i>K. rukwaensis</i> Seegers, 1995           |      | –              | –   | +              | –   | –              | –              | + <sup>T</sup> | –              | –              | +    | –              | –              | –   |
| <i>K. sjolandensi</i> Poll, 1967             |      | –              | –   | –              | –   | –              | –              | –              | –              | –              | –    | + <sup>T</sup> | –              | –   |
| <i>K. stappersii</i> Boulenger, 1915         |      | –              | –   | –              | –   | –              | + <sup>T</sup> | –              | –              | –              | –    | –              | –              | –   |
| <i>K. uluguru</i> Seegers, 1995              |      | –              | –   | –              | –   | –              | –              | –              | –              | + <sup>T</sup> | –    | –              | –              | –   |
| <i>K. wittei</i> Poll, 1944                  |      | –              | –   | –              | –   | + <sup>T</sup> | –              | –              | –              | –              | +    | –              | –              | –   |

**Table S2.** List of comparative *Kneria* species examined for the morphometric study. Ecoregions (ECRs): BM: Bangweulu–Mweru; ECB: Eastern Coastal Basin; KWA: Kwanza; LR: Lake Rukwa; PAN: Pangani; UC: upper Congo; UL: upper Lualaba; UZF: upper Zambezi Floodplains; and ZH: Zambezian Headwaters (sensu Thieme et al. [68]). F=Females and M=Males. ht = holotype; lt = lectotype; NS: Not Studied; plt(s): paralectotype(s); pt(s) = paratype(s); sp(s) = specimen(s); and st(s) = syntypes(s).

| Comparative species                          | Type locality (ECR)  | Specimens studied | Sex   |       |
|--|--|-------------------|-------|-------|
|  |  |                   | M (n) | F (n) |
| <i>K. angolensis</i> Steindachner, 1866      | Angola (KWA)   | NS                | 0     | 0     |
| <i>K. ansorgii</i> (Boulenger, 1910)         | Lucalla River. Kwanza basin. Angola (KWA)                  | sts               | 2     | 0     |
| <i>K. auriculata</i> (Pellegrin, 1905)       | Muza River. Zambezi basin. Mozambique (ZH)                 | lt + plts         | 3     | 0     |
| <i>K. katangae</i> Poll, 1976                | Mubale. lower Lufira basin. DRC (UL)                       | ht + pts          | 4     | 0     |
| <i>K. maydelli</i> Ladiges & Voelker, 1961   | Rua Cana. Cunene River system. Angola (ZH)                 | NS                | 0     | 0     |
| <i>K. paucisquamata</i> Poll & Stewart, 1975 | Luongo River. middle Luapula basin. DRC (BM)               | pts               | 5     | 3     |
| <i>K. polli</i> Trewavas, 1936               | Cuvo River system. coastal basin. Angola (UZF)             | sts               | 3     | 3     |
| <i>K. ruaha</i> Seegers, 1995                | Kisasa River. Ruaha drainage. Tanzania (ECB)               | NS                | 0     | 0     |
| <i>K. rukwaensis</i> Seegers, 1995           | Chiwanda River. Western Lake Rukwa drainage. Tanzania (LR) | NS                | 0     | 0     |
| <i>K. sjolandensi</i> Poll, 1967             | Cerilo. Cubal River. Angola (UZF)                          | NS                | 0     | 0     |
| <i>K. stappersii</i> Boulenger, 1915         | Lubumbashi. middle Luapula basin. DRC (BM)                 | (sts NI) sps      | 3     | 6     |
| <i>K. uluguru</i> Seegers, 1995              | Sombesi River. Upper Ruvu drainage. Tanzania (PAN)         | NS                | 0     | 0     |
| <i>K. wittei</i> Poll, 1944                  | Makala. Lukuga Basin. DRC (UC)                             | ht + sps          | 11    | 16    |



**Table S3.** Details on genetic samples used. The 81 newly generated sequences their access number begins with the code MN. cat. N°=catalogue number; F=Females, and M = Males, NS= Not Specified.

| Taxon on GenBank                              | Taxon in the tree                           | Museum cat. N°                   | GenBank Acc. N° | Sex |
|---|---|----------------------------------|-----------------|-----|
| <i>Kneria</i> sp. 'luansa-us'_24              | <i>Kneria</i> sp. luansa us 24_Paragenotype | RMCA_2016_038                    | MN594193        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_23              | <i>Kneria</i> sp. luansa-us 23_Paragenotype | RMCA_2016_038                    | MN594194        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_18              | <i>Kneria</i> sp. luansa us 18_Paragenotype | RMCA_2016_038                    | MN594195        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_11              | <i>Kneria</i> sp. luansa us 11_Paragenotype | RMCA_2016_038                    | MN594196        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_10              | <i>Kneria</i> sp. luansa us 10_Paragenotype | RMCA_2016_038                    | MN594197        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_2               | <i>Kneria</i> sp. luansa us 2_Paragenotype  | RMCA_2016_038                    | MN594198        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_219             | <i>Kneria</i> sp. luansa us 219             | RMCA_2016_038                    | MN594191        | NS  |
| <i>Kneria</i> sp. 'lutshipuka_(luansa)-ds'_58 | <i>Kneria</i> sp. luansa ds 58_Paragenotype | RMCA_2016_038                    | MN594202        | M   |
| <i>Kneria</i> sp. 'luthsipuka_(luansa)-ds'_70 | <i>Kneria</i> sp. luansa ds 70_Paragenotype | RMCA_2016_038                    | MN594203        | NS  |
| <i>Kneria</i> sp. 'luansa-us'_84              | <i>Kneria</i> sp. luansa us 84_Paragenotype | RMCA_2016_038                    | MN594204        | NS  |
| <i>Kneria</i> _cf_ansorgii_TFM39              | <i>Kneria</i> _cf_ansorgii_TFM39            | RMCA-2015-07                     | MN594176        | NS  |
| <i>Kneria</i> sp. 'lofoi-ds'_289              | <i>Kneria</i> sp. 'lofoi-ds'_289            | RMCA 2016-002-P-0309-0310-tag289 | MN594177        | F   |
| <i>Kneria</i> sp. 'lofoi-ds'_286              | <i>Kneria</i> sp. 'lofoi-ds'_286            | RMCA 2016-002-P-0315             | MN594178        | M   |
| <i>Kneria</i> sp. 'mwena-ds'_323              | <i>Kneria</i> sp. 'mwena-ds'_323            | RMCA 2016.002.P.0149-0151        | MN594179        | M   |
| <i>Kneria</i> sp. 'ditengwa'_36               | <i>Kneria</i> sp. 'ditengwa'_36             | RMCA-2015-07                     | MN594180        | NS  |
| <i>Kneria</i> _cf_ansorgii_TFM67              | <i>Kneria</i> _cf_ansorgii_TFM67            | RMCA-2015-07                     | MN594181        | NS  |
| <i>Kneria</i> sp. 'kililampuya'_9378          | <i>Kneria</i> sp. 'kililampuya'_9378        | RMCA-2012-031                    | MN594182        | NS  |
| <i>Kneria</i> sp. 'kililampuya'_9379          | <i>Kneria</i> sp. 'kililampuya'_9379        | RMCA-2012-031                    | MN594183        | NS  |
| <i>Kneria</i> sp. 'kabundji'_9374             | <i>Kneria</i> sp. 'kabundji'_9374           | RMCA-2012-031                    | MN594184        | NS  |
| <i>Kneria</i> sp. 'kabundji'_9376             | <i>Kneria</i> sp. 'kabundji'_9376           | RMCA-2012-031                    | MN594185        | NS  |

| Taxon on GenBank                                 | Taxon in the tree                                | Museum cat. N°                  | GenBank Acc. N° | Sex |
|--|--|---------------------------------|-----------------|-----|
| <i>Kneria</i> sp. 'lofoi-us'_5302                | <i>Kneria</i> sp. 'lofoi-us'_5302                | RMCA-2012-031-P-uncat           | MN594186        | F   |
| <i>Kneria</i> sp. 'lofoi-us'_5308                | <i>Kneria</i> sp. 'lofoi-us'_5308                | RMCA-2012-031                   | MN594187        | F   |
| <i>Kneria</i> sp. 'masansa-unif x tiger-us'_5457 | <i>Kneria</i> sp. 'masansa-unif x tiger-us'_5457 | RMCA-2012-031                   | MN594188        | M   |
| <i>Kneria</i> sp. 'masansa-uniform-us'_5466      | <i>Kneria</i> sp. 'masansa-uniform-us'_5466      | RMCA-2012-031                   | MN594189        | F   |
| <i>Kneria</i> sp. 'kamulonga'_9284               | <i>Kneria</i> sp. 'kamulonga'_9284               | RMCA-2012-031                   | MN594190        | NS  |
| <i>Kneria</i> sp. 'mwena-ds'_609                 | <i>Kneria</i> sp. 'mwena-ds'_609                 | RMCA_2016_038                   | MN594192        | NS  |
| <i>Kneria stappersii</i> _303                    | <i>Kneria stappersii</i> _303                    | RMCA_2016_038                   | MN594199        | NS  |
| <i>Kneria_cf_ansorgii</i> _TFM113                | <i>Kneria_cf_ansorgii</i> _TFM113                | RMCA-2015-07                    | MN594200        | NS  |
| <i>Kneria</i> sp. 'lutshipuka-ds'_822            | <i>Kneria</i> sp. 'lutshipuka-ds'_822            | RMCA 2015-006-P-0223            | MN594201        | M   |
| <i>Kneria stappersi</i> _st004                   | <i>Kneria stappersi</i> _st004                   | RMCA-2016-02                    | MN594205        | NS  |
| <i>Kneria stappersii</i> _st011                  | <i>Kneria stappersii</i> _st011                  | RMCA-2016-02                    | MN594206        | NS  |
| <i>Kneria</i> sp. 'luiji-us'_113                 | <i>Kneria</i> sp. 'luiji-us'_113                 | RMCA-B6-02                      | MN594207        | NS  |
| <i>Kneria</i> sp. 'luiji-us'_111                 | <i>Kneria</i> sp. 'luiji-us'_111                 | RMCA 2016.002.P.0127-0134_(111) | MN594208        | M   |
| <i>Kneria</i> sp. 'kasanga-us'_47                | <i>Kneria</i> sp. 'kasanga-us'_47                | RMCA 2016.002.P.0100-0108_47    | MN594209        | M   |
| <i>Kneria</i> sp. 'kasanga-us'_45                | <i>Kneria</i> sp. 'kasanga-us'_45                | RMCA 2016.002.P.0100-0108_45    | MN594210        | M   |
| <i>Kneria</i> sp. 'kasanga-us'_41                | <i>Kneria</i> sp. 'kasanga-us'_41                | RMCA 2016.002.P.0100-0108_41    | MN594211        | F   |
| <i>Kneria wittei</i> _MK00                       | <i>Kneria wittei</i> _MK00                       | RMCA-2015-07                    | MN594212        | NS  |
| <i>Kneria wittei</i> _LB01                       | <i>Kneria wittei</i> _LB01                       | RMCA-2015-07                    | MN594213        | NS  |
| <i>Kneria</i> sp. 'lutshipuka-ds'_79             | <i>Kneria</i> sp. 'lutshipuka-ds'_79             | RMCA-2015-06 (NM)               | MN594214        | M   |
| <i>Kneria</i> sp. 'lutshipuka-ds'_78             | <i>Kneria</i> sp. 'lutshipuka-ds'_78             | RMCA-2015-06 (NM)               | MN594215        | M   |
| <i>Kneria</i> sp. 'lutshipuka-ds'_77             | <i>Kneria</i> sp. 'lutshipuka-ds'_77             | RMCA-2015-06 (NM)               | MN594216        | M   |

| <b>Taxon on GenBank</b>                        | <b>Taxon in the tree</b>                       | <b>Museum cat. N°</b>     | <b>GenBank Acc. N°</b> | <b>Sex</b> |
|--|--|---------------------------|------------------------|------------|
| <i>Kneria</i> sp. 'lutshipuka-us'_55           | <i>Kneria</i> sp. 'lutshipuka-us'_55           | RMCA 2015-006-P-0216      | MN594217               | F          |
| <i>Kneria</i> sp. 'lutshipuka-us'_53           | <i>Kneria</i> sp. 'lutshipuka-us'_53           | RMCA-2015-06              | MN594218               | M          |
| <i>Kneria</i> sp. 'lutshipuka-us'_52           | <i>Kneria</i> sp. 'lutshipuka-us'_52           | RMCA-2015-06              | MN594219               | M          |
| <i>Kneria</i> sp. 'musipasi-us'_141            | <i>Kneria</i> sp. 'musipasi-us'_141            | RMCA 2015-006-P-0220      | MN594220               | F          |
| <i>Kneria</i> sp. 'musipasi-us'_144            | <i>Kneria</i> sp. 'musipasi-us'_144            | RMCA-2015-06              | MN594221               | M          |
| <i>Kneria</i> sp. 'lutshipuka-ds'_135          | <i>Kneria</i> sp. 'lutshipuka-ds'_135          | RMCA-2015-06 (NM)         | MN594222               | F          |
| <i>Kneria</i> sp. 'lutshipuka-ds'_137          | <i>Kneria</i> sp. 'lutshipuka-ds'_137          | RMCA-2015-06              | MN594223               | M          |
| <i>Kneria</i> sp. 'musipasi-us'_148            | <i>Kneria</i> sp. 'musipasi-us'_148            | RMCA 2015-006-P-0221      | MN594224               | F          |
| <i>Kneria</i> sp. 'lutshipuka-ds'_121          | <i>Kneria</i> sp. 'lutshipuka-ds'_121          | RMCA-2015-06              | MN594225               | M          |
| <i>Kneria</i> sp. 'masansa-uniform-us'_88      | <i>Kneria</i> sp. 'masansa-uniform-us'_88      | RMCA 2015-006-P-0218-0219 | MN594226               | F          |
| <i>Kneria</i> sp. 'masansa-tiger-us'_102       | <i>Kneria</i> sp. 'masansa-tiger-us'_102       | RMCA 2015-006-P-0217      | MN594227               | F          |
| <i>Kneria</i> sp. 'masansa-unif x tiger-us'_94 | <i>Kneria</i> sp. 'masansa-unif x tiger-us'_94 | RMCA-2015-06              | MN594228               | M          |
| <i>Kneria</i> sp. 'lutshipuka-ds'_116          | <i>Kneria</i> sp. 'lutshipuka-ds'_116          | RMCA-2015-06              | MN594229               | M          |
| <i>Kneria</i> sp. 'masansa-tiger-us'_99        | <i>Kneria</i> sp. 'masansa-tiger-us'_99        | RMCA-2015-06              | MN594230               | M          |
| <i>Kneria</i> sp. 'lutshipuka-ds'_115          | <i>Kneria</i> sp. 'lutshipuka-ds'_115          | RMCA-2015-06              | MN594231               | M          |
| <i>Kneria</i> sp. 'masansa-uniform-us'_90      | <i>Kneria</i> sp. 'masansa-uniform-us'_90      | RMCA 2015-006-P-0218-0219 | MN594232               | F          |
| <i>Kneria</i> sp. 'lofoi-bl'_285               | <i>Kneria</i> sp. 'lofoi-bl'_285               | RMCA-B5-06                | MN594233               | NS         |
| <i>Kneria</i> sp. 'lofoi-us'_4                 | <i>Kneria</i> sp. 'lofoi-us'_4                 | RMCA-2015-06 (NM)         | MN594234               | F          |
| <i>Kneria</i> sp. 'lofoi-us'_12                | <i>Kneria</i> sp. 'lofoi-us'_12                | RMCA-2015-06              | MN594235               | M          |
| <i>Kneria</i> sp. 'lofoi-us'_14                | <i>Kneria</i> sp. 'lofoi-us'_14                | RMCA-2015-06              | MN594236               | M          |
| <i>Kneria</i> sp. 'lofoi-us'_37                | <i>Kneria</i> sp. 'lofoi-us'_37                | RMCA-2015-06 (NE)         | MN594237               | F          |
| <i>Kneria</i> sp. 'lofoi-ds'_263               | <i>Kneria</i> sp. 'lofoi-ds'_263               | RMCA 2015-006-P-0642      | MN594238               | F          |
| <i>Kneria</i> sp. 'kalumengongo-ds'_0496       | <i>Kneria</i> sp. 'kalumengongo-ds'_0496       | RMCA-2015-05              | MN594239               | NS         |
| <i>Kneria</i> sp. 'lusinga-ds'_0466            | <i>Kneria</i> sp. 'lusinga-ds'_0466            | RMCA-2015-05              | MN594240               | NS         |
| <i>Kneria</i> sp. 'lutshipuka-us'_372          | <i>Kneria</i> sp. 'lutshipuka-us'_372          | RMCA-B2-31                | MN594241               | NS         |
| <i>Kneria</i> sp. 'mansha-ds'_3773             | <i>Kneria</i> sp. 'mansha-ds'_3773             | ZSM-PIS-044479            | MN594242               | NS         |

| Taxon on GenBank                     | Taxon in the tree                    | Museum cat. N° | GenBank Acc. N° | Sex |
|--------------------------------------|--------------------------------------|----------------|-----------------|-----|
| <i>Kneria</i> sp. 'lufubu'_3173      | <i>Kneria</i> sp. 'lufubu'_3173      | ZSM-PIS-044294 | MN594243        | NS  |
| <i>Kneria</i> sp. 'lunzua-ds'_3218   | <i>Kneria</i> sp. 'lunzua-ds'_3218   | ZSM-PIS-044279 | MN594244        | NS  |
| <i>Kneria</i> sp. 'lunzua-us'_3227   | <i>Kneria</i> sp. 'lunzua-us'_3227   | ZSM-PIS-044310 | MN594245        | NS  |
| <i>Kneria</i> sp. 'mukubwe-us'_3480  | <i>Kneria</i> sp. 'mukubwe-us'_3480  | ZSM-PIS-044381 | MN594246        | NS  |
| <i>Kneria</i> sp. 'mukubwe-ds'_3501  | <i>Kneria</i> sp. 'mukubwe-ds'_3501  | ZSM-PIS-044386 | MN594247        | NS  |
| <i>Kneria</i> sp. 'kalungwishi'_3520 | <i>Kneria</i> sp. 'kalungwishi'_3520 | ZSM-PIS-044386 | MN594248        | NS  |
| <i>Kneria</i> sp. 'ngona-us'_3535    | <i>Kneria</i> sp. 'ngona-us'_3535    | ZSM-PIS-044396 | MN594249        | NS  |
| <i>Kneria</i> sp. 'ngona-us'_3536    | <i>Kneria</i> sp. 'ngona-us'_3536    | ZSM-PIS-044396 | MN594250        | NS  |
| <i>Kneria</i> sp. 'ngona-bl'_3557    | <i>Kneria</i> sp. 'ngona-bl'_3557    | ZSM-PIS-044406 | MN594251        | NS  |
| <i>Kneria</i> sp. 'ngona-bl'_3559    | <i>Kneria</i> sp. 'ngona-bl'_3559    | ZSM-PIS-044406 | MN594252        | NS  |
| <i>Kneria</i> sp. 'itabu-us'_3603    | <i>Kneria</i> sp. 'itabu-us'_3603    | ZSM-PIS-044430 | MN594253        | NS  |
| <i>Kneria stappersii</i> _3706       | <i>Kneria stappersii</i> _3706       | ZSM-PIS-044455 | MN594254        | NS  |
| <i>Kneria stappersii</i> _3713       | <i>Kneria stappersii</i> _3713       | ZSM-PIS-044459 | MN594255        | NS  |
| <i>Kneria</i> sp. 'lulwe'_3904       | <i>Kneria</i> sp. 'lulwe'_3904       | ZSM-PIS-044538 | MN594256        | NS  |
| <i>Kneria uluguru</i> _1330          | <i>Kneria uluguru</i> _1330          | NS             | MN594257        | NS  |
| <i>Kneria uluguru</i> _1331          | <i>Kneria uluguru</i> _1331          | NS             | MN594258        | NS  |
| <i>Kneria maydelli</i> SAFW023-06    | <i>Kneria maydelli</i> SAFW023-06    | NS             | A146            | NS  |
| <i>Kneria maydelli</i> SAFW024-06    | <i>Kneria maydelli</i> SAFW024-06    | NS             | A147            | NS  |
| <i>Kneria maydelli</i> SAFW029-06    | <i>Kneria maydelli</i> SAFW029-06    | NS             | A219            | NS  |
| <i>Kneria maydelli</i> SAFW030-06    | <i>Kneria maydelli</i> SAFW030-06    | NS             | A232            | NS  |
| <i>Kneria</i> sp.                    | <i>Kneria</i> sp.                    | NS             |                 |     |
| <i>Parakneria cameronensis</i>       | <i>Parakneria cameronensis</i>       | NS             | NC_007891       | NS  |
| <i>Cromeria nilotica</i>             | <i>Cromeria nilotica</i>             | NS             | AP011560        | NS  |
| <i>Cromeria occidentalis</i>         | <i>Cromeria occidentalis</i>         | NS             | AP007275        | NS  |
| <i>Grasseichthys gabonensis</i>      | <i>Grasseichthys gabonensis</i>      | NS             | NC_007890       | NS  |
| <i>Chanos chanos</i>                 | <i>Chanos chanos</i>                 | NS             | NC 004693       | NS  |

**Table S4.** Characterisation of (i) the tubercles or lamellae in the opercular (T/L–OP) and (ii) the number of lamellae in the post–opercular organ (LPOP) of males; both as a function of (i) sampling period (S date / S season) and (ii) size, i.e. standard length ( $L_s$ ). T/L–OP: **(1)** without tubercles/lamellae; **(2)** with tubercles towards its outer region; and **(3)** with lamellae towards its outer region. UDP= undeveloped organ. Populations (from up– to downstream): KP1L= Luansa River on KP 1; KP2M = Milembwe, affluent river on KP 2; IntP1 = Intermediate plateau 1; IntP2= Intermediate plateau 2; IntP3= Intermediate plateau 3 (see Figure 2). Locality: us = upstream; ds = downstream, and S = Sampling. Sampling (S) date / season (see main text for details): DS=Dry season; and RS=Rainy season. C= Cold; H = Hot; L = Late and P=Peak. n=total number of specimens examined.

| Population/Species ID            | River             | Locality                   | S date / S season |    |    | $L_s$ (mm) | T/L–OP |   |   | LPOP   | n  |
|----------------------------------|-------------------|----------------------------|-------------------|----|----|------------|--------|---|---|--------|----|
|                                  |                   |                            | Date              | DS | RS |            | 1      | 2 | 3 |        |    |
| <b>K. sp. from lua–us (KP1L)</b> | Luansa            | us Kasompola Falls         | 19.09.2014        | H  |    | 37.3–46.3  | –      | – | 3 | 15     | 3  |
|                                  | Luansa            | us Kasompola Falls         | 20.08.2016        | H  |    | 39.2–40.0  | –      | 2 | – | 14–15  | 2  |
|                                  | Luansa            | us Kasompola Falls         | 21.09.2017        | H  |    | 39.4–50.2  | 5      | – | – | 16–19  | 5  |
|                                  | Luansa            | us Kasompola Falls         | 20.08.2016        | H  |    | 51.3–63.5  | 1      | – | 9 | 23–25  | 10 |
| K. sp. from lua–us (KP2M)        | Milembwe          | us Kasompola Falls         | 20.08.2016        | H  |    | 36.8–51.0  | 9      | 1 | – | 15–17  | 10 |
|                                  | Milembwe          | us Kasompola Falls         | 21.09.2017        | H  |    | 43.3–48.5  | 5      | – | – | 13–16  | 5  |
| K. sp. from lua–us (IntP1)       | Luansa            | us first Sanshifolo Falls  | 20.09.2017        | H  |    | 42.8–51.7  | 5      | 2 | 3 | 15–18  | 10 |
| K. sp. from lua–us (IntP2)       | Luansa            | us second Sanshifolo Falls | 23.08.2016        | H  |    | 36.8–55.9  | 3      | 4 | – | 15–17  | 7  |
|                                  | Luansa            | us second Sanshifolo Falls | 20.09.2017        | H  |    | 43.0–48.3  | 6      | – | 4 | 13–18  | 10 |
| K. sp. from lua–us (IntP3)       | Luansa            | us third Sanshifolo Falls  | 20.09.2017        | H  |    | 46.5–53.9  | 4      | 1 | – | 16–18  | 5  |
| K. sp. from lua–ds               | Luansa            | ds Sanshifolo Falls        | 22.08.2016        | H  |    | 42.2–57.0  | 1      | 4 | 4 | 16–23  | 9  |
|                                  | Luansa            | ds Sanshifolo Falls        | 19.09.2017        | H  |    | 52.3       | –      | – | 1 | 22     | 1  |
|                                  | Luansa            | ds Sanshifolo Falls        | 21.09.2017        | H  |    | 46.1       | 1      | – | – | UDP    | 1  |
| <i>K. stappersii</i>             | Lubumbashi        | Lubumbashi basin           | 08.1935           | H  |    | 37.3–45.0  | 1      | 5 | 1 | 12.–16 | 7  |
|                                  | Kamatete          | Lubumbashi basin           | 02.01.2016        |    | P  | 48.2–53.3  | 3      | – | – | 20     | 3  |
|                                  | Zoo de Lubumbashi | Lubumbashi basin           | 04.03.2020        |    | L  | 45.2–50.9  | 6      | – | – | 12–18  | 6  |
| <i>K. wittei</i>                 | Kamikua           | Lukuga basin               | 07.06.2015        | C  |    | 37.5–55.8  | 19     | – | – | 21–25  | 19 |
|                                  | Kaongo            | Lukuga basin               | 30.06.2015        | C  |    | 32.2–38.0  | 4      | – | – | 18–21  | 4  |

|          |              |            |   |           |    |   |   |       |    |
|----------|--------------|------------|---|-----------|----|---|---|-------|----|
| Djimbwe  | Lukuga basin | 30.06.2015 | C | 40.0–46.0 | 6  | – | – | 22–24 | 6  |
| Lubuye   | Lukuga basin | 07.06.2015 | C | 38.2–48.5 | 20 | – | – | 20–24 | 20 |
| Kyasombo | Lukuga basin | 07.06.2015 | C | 48.3–48.7 | 2  | – | – | 21–22 | 2  |

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**Table S5. (a)** Factor loadings for the first two axes of a PCA performed on four counts for all specimens examined (n=167). The most important values are in bold. **(b)** Results (p-values) of the MWU tests on two meristics, the seven remaining ones being invariable, between both sexes for the five most upstream populations of the Luansa River. **(c)** Results (p-values) of the MWU tests of the four meristics between both sexes for the (potential) species. ds = *K. sp.* 'lua-downstream' (= *K. luansaensis* sp. nov.); us = *K. sp.* 'lua-upstream' (= *K. maxi* sp. nov.); and wit = *K. wittei*. KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3 (Figure 2). ds: downstream, us: upstream. Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold; and highly significant values ( $p \leq 0.001$  after Bonferroni correction) in bold and underlined. -: for comparisons for which there is no variation in and between the compared populations/species.

| Variable                | a. PC Loadings  |                 | b. MWU tests         |                      |                    |                      |                    | c. MWU tests         |                     |                      |
|-------------------------|-----------------|-----------------|----------------------|----------------------|--------------------|----------------------|--------------------|----------------------|---------------------|----------------------|
|                         | PC 1            | PC 2            | KP1L                 | KP2M                 | IntP1              | IntP2                | IntP3              | us                   | ds                  | wit                  |
|                         |                 |                 | (M vs. F)<br>n=19x18 | (M vs. F)<br>n=15x15 | (M vs. F)<br>n=5x5 | (M vs. F)<br>n=12x13 | (M vs. F)<br>n=5x5 | (M vs. F)<br>n=56x56 | (M vs. F)<br>n=10x9 | (M vs. F)<br>n=11x16 |
| Lateral line scales     | <b>0.999790</b> | -0.019437       | 0.747897             | 0.723232             | 0.135100           | 0.179699             | 0.338270           | 0.372825             | 0.200053            | 0.940659             |
| Total pectoral fin rays | 0.019564        | <b>0.999450</b> | 0.590137             | –                    | 0.049535           | <b>0.001474</b>      | 0.220672           | <b>0.005834</b>      | 0.417831            | 0.142859             |
| Total pelvic fin rays   | 0.005613        | -0.023758       | –                    | –                    | –                  | –                    | –                  | –                    | –                   | 0.681153             |
| Total anal fin rays     | 0.001034        | 0.012435        | –                    | –                    | –                  | –                    | –                  | –                    | –                   | 0.002959             |

**Table S6.** Different combinations for which the MWU tests on the meristics were not performed due to few specimens being available ( $n \leq 4$ ).

| MWU tests not performed due to few specimens available                 |                                |
|--|--------------------------------|
| Population/species A   | Population/species B           |
| (i) Between males of all four (tentative) species                      |                                |
| <i>K. sp.</i> 'lua-upstream' (n=56)                                    | <i>K. stappersii</i> (n=3)     |
| <i>K. sp.</i> 'lua-downstream' (n=10)                                  | <i>K. stappersii</i> (n=3)     |
| (ii) Between Males (M) and Females (F) of all four (tentative) species |                                |
| <i>K. stappersii</i> (M) (n=3)   | <i>K. stappersii</i> (F) (n=6) |



**Table S7.** Results (p-values) of the Mann–Whitney U tests of the three meristics for the comparisons between the females of the six populations of the Luansa River. **(a)** Between each of the five most upstream populations (comparing the up– with each of the more downstream populations). However, comparisons were not possible between IntP1 vs. IntP3 due to significant size class differences (Table A10). **(b)** Between each of the five most upstream compared to the most downstream population. KP1L: Luansa River on the KP. loc 1; KP2M: Milembwe River on the KP. loc 2; IntP1: Intermediate plateau1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3; and ds: downstream (see Figure 2). Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold.

| <b>(a)</b><br><b>Variable</b> | <b>KP1L<br/>vs.<br/>KP2M<br/>n=18x15</b> | <b>KP1L<br/>vs.<br/>IntP1<br/>n=18x5</b> | <b>KP1L<br/>vs.<br/>IntP2<br/>n=18x13</b> | <b>KP1L<br/>vs.<br/>IntP3<br/>n=18x5</b> | <b>KP2M<br/>vs.<br/>IntP1<br/>n=15x5</b> | <b>KP2M<br/>vs.<br/>IntP2<br/>n=15x13</b> | <b>KP2M<br/>vs.<br/>IntP3<br/>n=15x5</b> | <b>IntP1<br/>vs.<br/>IntP2<br/>n=5x13</b> | <b>IntP1<br/>vs.<br/>IntP3<br/>n=5x5</b> | <b>IntP2<br/>vs.<br/>IntP3<br/>n=13x5</b> |
|-------------------------------|--|--|---|--|--|---|--|---|--|---|
| Lateral line scales           | 0.598697                                 | 0.039406                                 | 0.587160                                  | 0.244204                                 | 0.064957                                 | 0.330229                                  | 0.292524                                 | 0.012911                                  | 0.232824                                 | 0.058423                                  |
| Total pectoral fin rays       | 0.102471                                 | 0.338352                                 | 0.127604                                  | 0.864901                                 | –  | –   | 0.083265                                 | –   | 0.317311                                 | 0.106865                                  |
| Total pelvic fin rays         | 0.273323                                 | –  | –   | –  | 0.563703                                 | 0.351880                                  | 0.563703                                 | –   | –  | –   |

| <b>(b)</b><br><b>Variable</b> | <b>PL1<br/>vs.<br/>ds<br/>n=18x9</b> | <b>PL2<br/>vs.<br/>ds<br/>n=15x9</b> | <b>IntP1<br/>vs.<br/>ds<br/>n=5x9</b> | <b>IntP2<br/>vs.<br/>ds<br/>n=13x9</b> | <b>IntP3<br/>vs.<br/>ds<br/>n=5x9</b> |
|-------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|--|---------------------------------------|
| Lateral line scales           | <b>0.000033</b>                      | <b>0.000055</b>                      | <b>0.002417</b>                       | <b>0.000088</b>                        | <b>0.002500</b>                       |
| Total pectoral fin rays       | 0.731015                             | 0.061934                             | 0.272584                              | 0.081571                               | 0.925456                              |
| Total pelvic fin rays         |                                      | 0.438579                             |                                       |  |                                       |

**Table S8.** Results (p-values) of the Mann–Whitney U tests of the three meristics for the comparisons between the males of the six populations of the Luansa River. **(a)** Between each of the five most upstream populations (comparing the up– with each of the more downstream populations). However, comparisons were not possible between IntP1 vs. IntP3 due to significant size class differences (Table A10). **(b)** Between each of the five most upstream compared to the most downstream population. KP1L: Luansa River on the KP. loc 1; KP2M: Milembwe River on the KP. loc 2; IntP1: Intermediate plateau1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3; and ds: downstream (see Figure 2). Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold.

| <b>(a)</b><br>Variable  | KP1L<br>vs.<br>KP2M<br>n=19x15 | KP1L<br>vs.<br>IntP1<br>n=19x5 | KP1L<br>vs.<br>IntP2<br>n=19x12 | KP1L<br>vs.<br>IntP3<br>n=19x5 | KP2M<br>vs.<br>IntP1<br>n=15x5 | KP2M<br>vs.<br>IntP2<br>n=15x12 | KP2M<br>vs.<br>IntP3<br>n=15x5 | IntP1<br>vs.<br>IntP2<br>n=5x12 | IntP1<br>vs.<br>IntP3<br>n=5x5 | IntP2<br>vs.<br>IntP3<br>n=12x5 |
|-------------------------|--------------------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|---------------------------------|
| Lateral line scales     | 0.025017                       | 0.637698                       | 0.563221                        | 0.637470                       | 0.009473                       | 0.035786                        | 0.056941                       | 0.149609                        | 0.502335                       | 0.657506                        |
| Lamellae in the POP     | 0.485973                       | 0.370805                       | 0.713423                        | 0.099687                       | 0.725086                       | 0.768597                        | 0.313098                       | 0.521683                        | 0.201678                       | 0.111407                        |
| Total pectoral fin rays | 0.201941                       | 0.017660                       | 0.004956                        | 0.017660                       | 0.001516                       | <b>0.000744</b>                 | 0.001516                       | 0.950785                        | 1.000000                       | 0.950785                        |

| <b>(b)</b><br>Variable  | PL1<br>vs.<br>ds<br>n=19x10 | PL2<br>vs.<br>ds<br>n=15x10 | IntP1<br>vs.<br>ds<br>n=5x10 | IntP2<br>vs.<br>ds<br>n=12x10 | IntP3<br>vs.<br>ds<br>n=5x10 |
|-------------------------|-----------------------------|-----------------------------|------------------------------|-------------------------------|------------------------------|
| Lateral line scales     | <b>0.000017</b>             | <b>0.000039</b>             | 0.002043                     | <b>0.000083</b>               | 0.002081                     |
| Lamellae in the POP     | 0.559829                    | <b>0.000094</b>             | 0.010279                     | 0.003492                      | 0.006692                     |
| Total pectoral fin rays | 0.067251                    | 0.008829                    | 0.479501                     | 0.402784                      | 0.479501                     |

**Table S9.** Factor loadings for the first two axes of a PCA performed on four counts for all **(a)** females examined ( $n=87$ ) and **(b)** males examined ( $n=80$ ). The most important values are in bold. Results (p-values) of the Mann–Whitney U tests of these four meristics between **(c)** the females of the (potential) species and **(d)** males of (potential) species. ds = *K. sp.* ‘lua–downstream’ (= *K. maxi* sp. nov.) ; us = *K. sp.* ‘lua–upstream’ (= *K. luansaensis* sp. nov.) and wit = *K. wittei*. The number of counts remains 4, and not 5 with the addition of the number of lamellae for the males, as there is no variation in the total number of anal–fin rays in males only. Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold; and highly significant values ( $p \leq 0.001$  after Bonferroni correction) in bold and underlined. –: for comparisons for which there is no variation in and between the compared populations/species.

| Variables               | a. PC Loadings  |                | b. PC Loadings  |                 | c. MWU tests (females) |                        |                        |                        |          | d. MWU tests (males) |                        |                 |
|-------------------------|-----------------|----------------|-----------------|-----------------|------------------------|------------------------|------------------------|------------------------|----------|----------------------|------------------------|-----------------|
|                         | PC 1            | PC 2           | PC 1            | PC 2            | us                     | us                     | us                     | ds                     | ds       | us                   | us                     | ds              |
|                         |                 |                |                 |                 | vs.                    | vs.                    | vs.                    | vs.                    | vs.      | vs.                  | vs.                    | vs.             |
|                         |                 |                |                 |                 | wit                    | sta                    | ds                     | wit                    | sta      | wit                  | ds                     | wit             |
|                         |                 |                |                 |                 | n=56x16                | n=56x6                 | n=56x9                 | n=9x16                 | n=9x6    | n=56x11              | n=56x10                | n=10x11         |
| Lamellae in the POP     | /               | /              | 0.325470        | <b>0.945390</b> | /                      | /                      | /                      | /                      | /        | <b>0.001417</b>      | <b>0.004750</b>        | 0.521513        |
| Lateral line scales     | <b>0.999710</b> | –0.021354      | <b>0.945390</b> | –0.325630       | 0.496899               | <b>0.000060</b>        | <u><b>0.000002</b></u> | <u><b>0.000044</b></u> | 0.434059 | 0.779106             | <u><b>0.000001</b></u> | <b>0.000119</b> |
| Total anal fin rays     | 0.002046        | –0.004584      | –               | –               | 0.273323               | –                      | –                      | –                      | –        | –                    | –                      | –               |
| Total pectoral fin rays | 0.023050        | <b>0.97934</b> | 0.017066        | 0.011198        | 0.169918               | <u><b>0.000001</b></u> | 0.150033               | 0.838257               | 0.024745 | 0.219536             | 0.398859               | 0.805541        |
| Total pelvic fin rays   | 0.006052        | –0.20104       | 0.004503        | –0.009036       | <b>0.000286</b>        | 0.743421               | 0.688500               | 0.108810               | –        | <b>0.001303</b>      | –                      | 0.166534        |

**Table S10. (a)** Different combinations used for the Mann–Whitney U (MWU) tests on the measurements and their size classes; **(b)** different combinations for which the MWU tests were not performed due to size differences or few specimens being available.

| <b>(a) MWU tests performed between similar size classes</b>                          |                                      |                                    |   |
|--|--------------------------------------|------------------------------------|---|
| <b>Population/species A</b>  | <b>Population/species B</b>          | <b><math>L_s</math> P &gt; 0.5</b> | <b><math>L_s</math> ranges compared between A vs. B (mm <math>L_s</math>)</b> |
| <b>(i) Between females of the five most upstream populations of the Luansa River</b> |                                      |                                    |   |
| KP1L (n=11)  | KP2M (n=13)                          | 0.582047                           | 38.5–60.0 vs. 39.6–60.6   |
| KP1L (n=18)  | IntP1 (n=5)                          | 0.601756                           | 38.5–70.0 vs. 56.0–65.5   |
| KP1L (n=10)  | IntP2 (n=11)                         | 0.597288                           | 38.5–58.8 vs. 38.4–58.8   |
| KP1L (n=18)  | IntP3 (n=5)                          | 0.709320                           | 38.5–70.0 vs. 50.9–69.2   |
| KP2M (n=8)   | IntP1 (n=5)                          | 0.509486                           | 49.0–68.4 vs. 56.0–65.5   |
| KP2M (n=15)  | IntP2 (n=13)                         | 0.799991                           | 39.6–68.4 vs. 38.4–65.8   |
| KP2M (n=8)   | IntP3 (n=5)                          | 0.883618                           | 49.0–68.4 vs. 50.9–69.2   |
| IntP1 (n=5)  | IntP2 (n=5)                          | 0.754023                           | 56.0–65.5 vs. 52.8–65.8   |
| IntP2 (n=7)  | IntP3 (n=5)                          | 0.569765                           | 49.0–65.4 vs. 50.9–69.2   |
| <b>(ii) Between females of the four (tentative) species</b>                          |                                      |                                    |   |
| <i>K. sp.</i> 'lua-upstream' (n=40)  | <i>K. wittei</i> (n=16)              | 0.913337                           | 38.4–60.6 vs. 34.2–59.8   |
| <i>K. sp.</i> 'lua-upstream' (n=56)  | <i>K. stappersii</i> (n=6)           | 0.924123                           | 38.4–70.0 vs. 40.6–62.4   |
| <i>K. sp.</i> 'lua-upstream' (n=56)  | <i>K. sp.</i> 'lua-downstream' (n=9) | 0.909266                           | 38.4–70.0 vs. 40.2–63.8   |
| <i>K. sp.</i> 'lua-downstream' (n=7)   | <i>K. wittei</i> (n=15)              | 0.805127                           | 40.2–62.0 vs. 40.7–62.5   |
| <b>(iii) Between males of the five most upstream populations of the Luansa River</b> |                                      |                                    |   |
| KP1 (n=9)  | KP2 (n=15)                           | 0.857997                           | 37.3–50.2 vs. 35.7–49.6   |
| KP1 (n=19)   | Int1 (n=5)                           | 0.749068                           | 37.3–63.0 vs. 48.0–51.7   |
| KP1 (n=13)   | Int2 (n=12)                          | 0.703389                           | 37.3–57.3 vs. 36.8–55.9   |
| KP1 (n=19)   | Int3 (n=5)                           | 0.803526                           | 37.3–63.0 vs. 46.5–53.9   |
| KP2 (n=11)   | Int2 (n=11)                          | 0.532748                           | 35.7–49.6 vs. 40.2–55.9   |
| <b>(iv) Between males of the four (tentative) species</b>                            |                                      |                                    |   |

|   |  |                                    |  |
|---|--|------------------------------------|--|
| <i>K. sp.</i> 'lua-upstream' (n=56)   | <i>K. wittei</i> (n=11)                  | 0.932556                           | 35.7–63.0 vs. 32.9–55.8  |
| <i>K. sp.</i> 'lua-upstream' (n=28)   | <i>K. sp.</i> 'lua-downstream' (n=9)     | 0.607724                           | 46.3–53.9 vs. 46.1–57.0  |
| <b>(v) Between Males (M) and Females (F) of the five most upstream populations of the Luansa River</b>      |  |                                    |  |
| KP1 (M) (n=19)  | KP1 (F) (n=13)                           | 0.631500                           | 37.3–63.0 vs. 38.5–70.0  |
| KP2 (M) (n=9)   | KP2 (F) (n=12)                           | 0.522432                           | 35.7–49.7 vs. 39.6–68.4  |
| IntP2 (M) (n=12)  | IntP2 (F) (n=10)                         | 0.509652                           | 36.7–55.9 vs. 38.4–65.8  |
| <b>(vi) Between Males (M) and Females (F) of the four (tentative) species</b>                               |  |                                    |  |
| <i>K. sp.</i> 'lua-upstream' (M) (n=37)   | <i>K. sp.</i> 'lua-upstream' (F) (n=39)  | 0.509299                           | 35.7–63.0 vs. 38.4–70.0  |
| <i>K. sp.</i> 'lua-downstream' (M) (n=10)   | <i>K. sp.</i> 'lua-downstream' (F) (n=6) | 0.587594                           | 42.2–57.0 vs. 40.2–63.8  |
| <i>K. wittei</i> (M) (n=10)   | <i>K. wittei</i> (F) (n=10)              | 0.832689                           | 32.9–55.8 vs. 34.2–59.8  |
| <b>(b) MWU tests not performed due to few specimens available or size differences</b>                       |  |                                    |  |
| <b>Population/species A</b>   | <b>Population/species B</b>              | <b><math>L_s</math> P &gt; 0.5</b> | <b><math>L_s</math> range compared species A vs. B (mm <math>L_s</math>)</b> |
| <b>(i) Between females of the five most upstream populations of the Luansa River</b>                        |  |                                    |  |
| IntP1 (n=5)   | IntP3 (n=5)                              | size differences                   | 56.0–65.5 vs. 50.9–69.2  |
| <b>(ii) Between Females of all four (tentative) species</b>   |  |                                    |  |
| <i>K. sp.</i> 'lua-downstream' (n=8)  | <i>K. stappersii</i> (n=6)               | size differences                   | 40.2–62.5 vs. 40.6–62.4  |
| <b>(iii) Between males of the five most upstream populations of the Luansa River</b>                        |  |                                    |  |
| KP2M (n=15)   | IntP1 (n=5)                              | size differences                   | 35.7–49.6 vs. 48.0–51.7  |
| KP2M (n=15)   | IntP3 (n=5)                              | size differences                   | 35.7–49.6 vs. 46.5–53.9  |
| IntP1 (n=5)   | IntP2 (n=12)                             | size differences                   | 48.0–51.7 vs. 36.8–55.9  |
| IntP2 (n=12)  | IntP3 (n=5)                              | size differences                   | 36.8–55.9 vs. 46.5–53.9  |
| <b>(iv) Between males of all four (tentative) species</b>   |  |                                    |  |
| <i>K. sp.</i> 'lua-upstream' (n=20)   | <i>K. stappersii</i> (n=3)               | few specimens                      | 48.0–60.3 vs. 48.2–53.3  |
| <i>K. sp.</i> 'lua-downstream' (n=10)   | <i>K. wittei</i> (n=9)                   | size differences                   | 42.2–57.0 vs. 32.9–50.8  |
| <i>K. sp.</i> 'lua-downstream' (n=10)   | <i>K. stappersii</i> (n=3)               | size differences                   | 42.2–57.0 vs. 48.2–53.3  |
| <b>(v) Between Males (M) and Females (F) of the most five most upstream populations of the Luansa River</b> |  |                                    |  |
| IntP1 (M) (n=5)   | IntP1 (F) (n=5)                          | size differences                   | 48.0–51.7 vs. 56.0–65.5  |
| IntP3 (M) (n=5)   | IntP3 (F) (n=5)                          | size differences                   | 46.5–53.9 vs. 50.9–69.2  |
| <b>(vi) Between Males (M) and Females (F) of all four (tentative) species</b>                               |  |                                    |  |
| <i>K. stappersii</i> (M) (n=3)  | <i>K. stappersii</i> (n=6)               | few specimens                      | 48.2–53.3 vs. 40.6–62.4  |

**Table S11. (a)** Factor loadings for the second and third axes of a PCA performed with 31 measurements for all females examined (n=87). The most important values are in bold. **(b)** Factor loadings for the second and third axes of a PCA performed with 31 measurements for all females of *K. stappersii* and *K. sp. 'lua-downstream'* (= *K. maxi*) (n=15) only. **(c)** Results (p-values) of the MWU tests for the 31 measurements (expressed as percentages) between females of all four (tentative) species. ds = *K. sp. 'lua-downstream'* (= *K. maxi* sp. nov.); sta = *K. stappersii*; us = *K. sp. 'lua-upstream'* (= *K. luansaensis* sp. nov.; and wit = *K. wittei*. Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold; and highly significant values ( $p \leq 0.001$  after Bonferroni correction) in bold and underlined.

| Variable                 | a. PC Loadings   |                 | b. PC Loadings   |                  | c. MWU tests (females) |                 |                 |          |
|--------------------------|------------------|-----------------|------------------|------------------|------------------------|-----------------|-----------------|----------|
|                          | PC 2             | PC 3            | PC 2             | PC 3             | us                     | us              | us              | ds       |
|                          |                  |                 |                  |                  | vs.                    | vs.             | vs.             | vs.      |
|                          |                  |                 |                  |                  | wit                    | sta             | ds              | wit      |
|                          |                  |                 |                  |                  | n=40x16                | n=56x6          | n=56x9          | n=7x15   |
| Standard length          | 0.039725         | -0.108430       | 0.028358         | -0.017646        | 0.913337               | 0.924123        | 0.909266        | 0.805127 |
| Post-orbital distance    | 0.231740         | 0.046924        | -0.131180        | -0.106420        | 0.016661               | 0.034085        | 0.028943        | 0.915792 |
| Interorbital distance    | -0.027401        | 0.017228        | -0.094881        | -0.039374        | 0.086530               | 0.090934        | 0.094625        | 0.860116 |
| Head height              | 0.046491         | 0.077014        | 0.013066         | -0.166720        | 0.927743               | 0.070367        | 0.305054        | 0.417571 |
| Head width               | -0.219340        | 0.230400        | 0.089209         | <b>-0.264140</b> | <b>0.000077</b>        | <b>0.000063</b> | <b>0.000011</b> | 0.015019 |
| Snout length             | -0.229610        | 0.208830        | <b>-0.272110</b> | <b>0.324340</b>  | 0.000307               | 0.253087        | 0.000331        | 0.549059 |
| Mouth width              | <b>-0.368740</b> | <b>0.270380</b> | -0.034685        | <b>-0.321740</b> | <u><b>0.000001</b></u> | <b>0.000115</b> | <b>0.000013</b> | 0.274567 |
| Eye diameter             | -0.081910        | -0.161390       | <b>-0.368020</b> | 0.176680         | <b>0.000017</b>        | 0.025980        | 0.068206        | 0.037574 |
| Head length              | 0.046863         | 0.034157        | -0.022227        | 0.101900         | 0.024515               | 0.520312        | 0.296186        | 0.015019 |
| Pre-dorsal distance      | 0.053284         | -0.127690       | 0.031010         | 0.015659         | 0.106488               | 0.019631        | 0.004939        | 0.000484 |
| Pectoral-pelvic distance | -0.092483        | -0.133960       | 0.007340         | -0.048703        | 0.001926               | 0.004607        | 0.000383        | 0.169266 |
| Pectoral-anal distance   | -0.040822        | -0.147040       | 0.062846         | -0.062144        | <b>0.000015</b>        | 0.010128        | 0.050428        | 0.129634 |
| Post-dorsal distance     | 0.005241         | -0.064630       | 0.051012         | 0.004391         | 0.676570               | 0.001308        | 0.000266        | 0.004306 |
| Pre-pelvic distance      | 0.009627         | -0.066213       | -0.030425        | -0.072976        | 0.799560               | 0.040598        | 0.002374        | 0.006650 |
| Pre-anal distance        | 0.020093         | -0.108440       | 0.011996         | -0.020693        | 0.091656               | 0.617075        | 0.203175        | 0.417571 |

|                         |                  |                  |                  |                  |                        |                 |                        |                 |
|-------------------------|------------------|------------------|------------------|------------------|------------------------|-----------------|------------------------|-----------------|
| Pre-pectoral distance   | 0.110020         | -0.026258        | -0.165240        | 0.194650         | 0.000629               | 0.011610        | 0.055069               | 0.000629        |
| Body height             | <b>-0.451430</b> | <b>-0.333900</b> | <b>0.558280</b>  | -0.075564        | <u><b>0.000000</b></u> | <b>0.000115</b> | 0.062695               | 0.002167        |
| Body width              | -0.225730        | 0.204390         | 0.013228         | -0.037546        | 0.118789               | <b>0.000063</b> | <u><b>0.000002</b></u> | <b>0.000215</b> |
| Caudal peduncle height  | 0.187260         | 0.204710         | -0.105230        | -0.100970        | 0.001096               | 0.198544        | 0.804974               | 0.061762        |
| Caudal peduncle length  | -0.012312        | -0.149240        | -0.064493        | 0.242890         | 0.059246               | 0.317287        | 0.002373               | 0.217357        |
| Dorsal height           | 0.129040         | 0.022095         | 0.217840         | <b>0.359670</b>  | <b>0.000007</b>        | 0.600407        | 0.114918               | 0.052564        |
| Pelvic length           | 0.140910         | -0.051561        | 0.126100         | 0.112450         | <b>0.000112</b>        | 0.924126        | 0.436139               | 0.031559        |
| Pectoral length         | -0.074019        | 0.007791         | 0.194110         | <b>0.281690</b>  | 0.730394               | 0.001815        | 0.009792               | 0.072255        |
| Upper caudal fin lobe   | 0.059811         | -0.042991        | 0.221450         | <b>0.267150</b>  | 0.553182               | 0.383292        | 0.319943               | 0.971884        |
| Lower caudal fin lobe   | <b>0.264480</b>  | -0.022678        | -0.053424        | 0.215250         | 0.033053               | 0.000662        | 0.138473               | 0.804911        |
| Dorsal fin base width   | 0.247830         | 0.082718         | 0.117360         | -0.207630        | <u><b>0.000003</b></u> | 0.867629        | 0.065417               | 0.129634        |
| Pelvic fin base width   | 0.249680         | -0.186390        | -0.067193        | -0.147140        | 0.002110               | 0.003675        | 0.001418               | 0.860116        |
| Pectoral fin base width | -0.173520        | <b>0.421230</b>  | 0.089463         | <b>-0.273540</b> | 0.884636               | <b>0.000224</b> | <b>0.000145</b>        | 0.001708        |
| Anal fin base width     | <b>0.325790</b>  | <b>0.403460</b>  | <b>-0.449300</b> | -0.156240        | <b>0.000104</b>        | 0.013279        | 0.159865               | 0.004306        |
| Pelvic-anal distance    | -0.026171        | <b>-0.313220</b> | 0.097038         | 0.089838         | <b>0.000150</b>        | 0.365592        | 0.250509               | 0.008208        |
| Anal-caudal distance    | 0.065141         | -0.054819        | -0.011735        | 0.036890         | 0.457103               | 0.460451        | 0.676050               | 0.860116        |

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**Table S12.** Results (p-values) of the MWU tests of the 31 measurements for the comparisons between the females of the six populations of the Luansa River. **(a)** Between each of the most five most upstream populations (comparing the up- with each of the more downstream populations). However, comparisons were not possible between IntP1 vs. IntP3 due to significant size class differences (Table A10). **(b)** Between each of the five most upstream compared to the most downstream population. KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: Intermediate plateau1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3 (Figure 2). ds: downstream, us: upstream. Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold.

| (a)<br>Variable          | KP1L            | KP1L            | KP1L             | KP1L            | KP2M           | KP2M             | KP2M           | IntP1          | IntP2          |
|--------------------------|-----------------|-----------------|------------------|-----------------|----------------|------------------|----------------|----------------|----------------|
|                          | vs.             | vs.             | vs.              | vs.             | vs.            | vs.              | vs.            | vs.            | vs.            |
|                          | KP2M<br>n=11x13 | IntP1<br>n=18x5 | IntP2<br>n=10x11 | IntP3<br>n=18x5 | IntP1<br>n=8x5 | IntP2<br>n=15x13 | IntP3<br>n=8x5 | IntP2<br>n=5x5 | IntP3<br>n=7x5 |
| Standard length          | 0.582047        | 0.601756        | 0.597288         | 0.709320        | 0.509486       | 0.799991         | 0.883618       | 0.754023       | 0.569765       |
| Post-orbital distance    | 0.839309        | 0.881498        | 0.887964         | 0.654721        | 0.107347       | 0.729691         | 0.187684       | 0.347208       | 0.935283       |
| Interorbital distance    | 0.434131        | 0.025348        | 0.090918         | 0.025348        | 0.019173       | 0.001600         | 0.241567       | 0.916815       | 0.007372       |
| Head height              | 0.542967        | 0.004611        | 0.20497          | 0.412163        | 0.040425       | 0.017675         | 1.000000       | 0.601509       | 0.167466       |
| Head width               | 0.139574        | 0.025348        | 0.724771         | 0.550985        | 0.078984       | 0.695387         | 1.000000       | 0.075801       | 0.464903       |
| Snout length             | 0.339097        | 0.233039        | 0.290847         | 0.136038        | 0.769698       | 0.871913         | 0.660550       | 0.601509       | 0.684744       |
| Mouth width              | 0.505239        | 0.179713        | 0.139201         | 0.940584        | 1.000000       | 0.008079         | 0.187684       | 0.464703       | 0.807541       |
| Eye diameter             | 0.098407        | 0.020824        | 0.48118          | 0.765538        | 0.464215       | 0.533908         | 0.305508       | 0.601509       | 0.371752       |
| Head length              | 0.087428        | 0.017073        | 0.573202         | 0.233039        | 0.078984       | 0.564739         | 0.379776       | 0.174526       | 0.028352       |
| Pre-dorsal distance      | 0.930747        | 0.296718        | 0.091023         | 0.179713        | 0.558185       | 0.122786         | 0.883618       | 0.754023       | 0.569765       |
| Pectoral-pelvic distance | 0.022109        | 0.002244        | 0.290847         | 0.940584        | 0.012827       | 0.240129         | 0.040425       | 0.009024       | 0.291153       |
| Pectoral-anal distance   | 0.468939        | 0.009088        | 0.259876         | 0.881498        | 0.019173       | 0.240129         | 1.000000       | 0.009024       | 0.807541       |
| Post-dorsal distance     | 0.155770        | 0.456057        | 0.481322         | 0.296718        | 0.558185       | 0.020003         | 0.305508       | 0.916815       | 0.684744       |
| Pre-pelvic distance      | 0.139574        | 0.000796        | 0.024236         | 0.136038        | 0.003415       | 0.695387         | 0.187684       | 0.009024       | 0.569765       |
| Pre-anal distance        | 0.087428        | 0.044172        | 0.778196         | 0.765595        | 0.005414       | 0.005321         | 0.241567       | 0.174526       | 0.935283       |
| Pre-pectoral distance    | 0.155770        | 0.156725        | 0.007454         | 0.073639        | 1.000000       | 0.012054         | 0.057041       | 0.174526       | 0.569765       |



|                          |          |          |          |          |          |          |          |          |          |
|--------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Body height              | 0.087428 | 0.062408 | 0.024236 | 0.101051 | 0.005414 | 0.18923  | 0.028109 | 0.028281 | 0.018534 |
| Body width               | 0.111104 | 0.179606 | 0.832636 | 0.765538 | 0.040425 | 0.447146 | 0.464215 | 0.347208 | 0.371752 |
| Caudal peduncle height   | 0.884836 | 0.456057 | 0.231267 | 0.086471 | 0.143236 | 0.321977 | 0.769698 | 0.754023 | 0.167466 |
| Caudal peduncle length   | 0.976885 | 0.156622 | 0.245126 | 0.550887 | 0.107347 | 0.112004 | 0.66055  | 0.601509 | 0.464903 |
| Dorsal height            | 0.468939 | 0.156725 | 0.778196 | 0.263553 | 1.000000 | 0.47522  | 0.464215 | 0.754023 | 0.569765 |
| Pelvic length            | 0.192380 | 0.371094 | 0.888000 | 0.709388 | 0.464215 | 0.394099 | 1.000000 | 0.174526 | 0.684744 |
| Pectoral length          | 0.505239 | 0.881498 | 0.204970 | 0.030655 | 0.187684 | 0.504168 | 0.464215 | 0.916815 | 0.371752 |
| Upper caudal fin lobe    | 0.172448 | 0.610578 | 0.210001 | 0.844716 | 0.66055  | 0.944912 | 0.558185 | 0.916815 | 0.935283 |
| Lower caudal fin lobe    | 0.192380 | 0.073639 | 0.526237 | 0.615303 | 1.000000 | 0.908316 | 0.838257 | 0.754023 | 0.73244  |
| Dorsal fin base length   | 0.223530 | 0.550985 | 0.204970 | 0.601845 | 0.769698 | 0.835758 | 0.379776 | 0.174526 | 0.684744 |
| Pelvic fin base length   | 0.839309 | 0.910936 | 0.121336 | 0.002862 | 1.000000 | 0.240129 | 0.241567 | 0.601509 | 0.122881 |
| Pectoral fin base length | 0.582047 | 0.205119 | 0.016657 | 0.412279 | 0.187684 | 0.279015 | 1.000000 | 0.754023 | 0.088159 |
| Anal fin base length     | 0.930747 | 0.502335 | 0.159026 | 0.025348 | 0.143236 | 0.122786 | 0.379776 | 0.117186 | 0.061819 |
| Pelvic anal distance     | 0.400864 | 1.000000 | 0.832689 | 0.456057 | 0.187684 | 0.068824 | 0.241567 | 0.464703 | 0.371752 |
| Anal–caudal distance     | 0.930747 | 0.550985 | 0.647050 | 0.263553 | 0.558185 | 0.36904  | 0.558185 | 0.601509 | 0.371752 |

|                          | PL1             | PL2             | IntP1    | IntP2           | IntP3    |
|--------------------------|-----------------|-----------------|----------|-----------------|----------|
| (b)                      | vs.             | vs.             | vs.      | vs.             | vs.      |
| Variable                 | ds              | ds              | ds       | ds              | ds       |
|                          | n=16x9          | n=14x9          | n=5x7    | n=12x9          | n=5x7    |
| Standard length          | 0.734046        | 0.614295        | 0.569765 | 0.569672        | 0.684744 |
| Post-orbital distance    | 0.031451        | 0.256840        | 0.088159 | 0.046535        | 0.291153 |
| Interorbital distance    | 0.089430        | 0.019766        | 0.807541 | 0.521891        | 0.007372 |
| Head height              | 0.954853        | 0.899741        | 0.088159 | 0.046606        | 0.935283 |
| Head width               | <b>0.000094</b> | <b>0.000157</b> | 0.018534 | <b>0.000290</b> | 0.028352 |
| Snout length             | 0.002695        | <b>0.000842</b> | 0.028352 | 0.015681        | 0.122881 |
| Mouth width              | <b>0.000058</b> | 0.003069        | 0.007372 | <b>0.000380</b> | 0.004484 |
| Eye diameter             | 0.079197        | 0.147277        | 0.935283 | 0.075243        | 0.061819 |
| Head length              | 0.571300        | 0.147377        | 0.018534 | 0.226995        | 0.371752 |
| Pre-dorsal distance      | 0.650613        | 0.003069        | 0.088159 | <b>0.000220</b> | 0.011830 |
| Pectoral-pelvic distance | <b>0.000362</b> | 0.004587        | 0.935283 | 0.004474        | 0.007372 |
| Pectoral-anal distance   | 0.036197        | 0.231351        | 0.042358 | 0.010516        | 0.061819 |
| Post-dorsal distance     | 0.027249        | <b>0.000094</b> | 0.007372 | 0.003571        | 0.007372 |
| Pre-pelvic distance      | <b>0.000187</b> | 0.005576        | 0.291153 | 0.027590        | 0.042358 |
| Pre-anal distance        | 0.192877        | 0.009802        | 0.088159 | 0.669816        | 0.807541 |
| Pre-pectoral distance    | 0.036197        | 0.004587        | 0.028352 | 0.943345        | 0.569765 |
| Body height              | 0.020278        | 0.412831        | 0.004484 | 0.943345        | 0.028352 |
| Body width               | <b>0.000046</b> | <b>0.000072</b> | 0.004484 | <b>0.000124</b> | 0.004484 |
| Caudal peduncle height   | 0.496906        | 0.256840        | 0.935283 | 0.886974        | 0.371752 |
| Caudal peduncle length   | 0.017396        | 0.003069        | 0.223226 | 0.046606        | 0.122881 |
| Dorsal height            | 0.308180        | 0.088974        | 0.088159 | 0.355555        | 0.122881 |
| Pelvic length            | 0.395766        | 0.088974        | 0.807541 | 0.669816        | 0.569765 |

|                          |                 |          |          |                 |          |
|--------------------------|-----------------|----------|----------|-----------------|----------|
| Pectoral length          | 0.212947        | 0.003069 | 0.464903 | 0.008552        | 0.061819 |
| Upper caudal fin lobe    | 0.089243        | 0.850107 | 0.291153 | 0.831171        | 0.291153 |
| Lower caudal fin lobe    | 0.100632        | 0.185878 | 0.061819 | 0.393769        | 0.684221 |
| Dorsal fin base length   | 0.141033        | 0.147377 | 0.122881 | 0.117943        | 0.684744 |
| Pelvic fin base length   | <b>0.001847</b> | 0.014020 | 0.018534 | 0.008552        | 0.935283 |
| Pectoral fin base length | <b>0.001025</b> | 0.009802 | 0.004484 | <b>0.000380</b> | 0.028352 |
| Anal fin base length     | 0.126371        | 0.284216 | 0.569765 | 0.135594        | 0.569765 |
| Pelvic–anal distance     | 0.202644        | 0.801060 | 0.042358 | 0.064641        | 0.569765 |
| Anal–caudal distance     | 0.461743        | 0.528734 | 0.684744 | 0.722339        | 0.935283 |

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**Table S13. (a)** Factor loadings for the second and third axes of a PCA performed with 34 measurements on all males examined (n =80). The most important values are in bold. **(b)** Results (p-values) of the MWU tests of the 34 measurements (expressed as percentages) for the comparisons between the males of the five most upstream populations of the Luansa River. KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3 (Figure 2). ds: downstream, us: upstream. Comparisons, indeed, were not possible between the two remaining populations, i.e. P2 vs. Int1, P2 vs. Int3, Int1 vs. Int2, Int1 vs. Int3, IntP2 vs. IntP3 due to significant size class differences (Table A10). **(c)** Results (p-values) of the MWU tests of the 34 measurements (expressed as percentages) for the comparisons between the males. ds = *K. sp.* 'lua-downstream' (= *K. maxi* sp. nov.); us= *K. sp.* 'lua-upstream' (= *K. luansaensis* sp. nov.); and wit = *K. wittei*. Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold; and highly significant values ( $p \leq 0.001$  after Bonferroni correction) in bold and underlined.

| Variable                               | a. PC Loadings  |                  | b. MWU tests (males) |                |                 |                |                 |
|--|-----------------|------------------|----------------------|----------------|-----------------|----------------|-----------------|
|  | PC 2            | PC 3             | KP1                  | KP1            | KP1             | KP1            | KP2             |
|  |                 |                  | vs.                  | vs.            | vs.             | vs.            | vs.             |
|  |                 |                  | KP2<br>n=9x15        | Int1<br>n=19x5 | Int2<br>n=13x12 | Int3<br>n=19x5 | Int2<br>n=11x11 |
| Standard length                        | 0.002588        | -0.089230        | 0.857997             | 0.749068       | 0.703389        | 0.803526       | 0.532748        |
| Post-orbital distance                  | -0.202930       | -0.010310        | 0.101051             | 0.145068       | 0.414562        | 0.455451       | 0.308770        |
| Interorbital distance                  | -0.087527       | <b>0.302460</b>  | 0.114072             | 0.094837       | 0.827763        | 0.858955       | 0.450161        |
| Head height                            | -0.046300       | 0.092958         | 0.004621             | 0.002520       | 0.301388        | 0.302688       | 0.013800        |
| Head width                             | 0.078976        | 0.177890         | 0.015738             | 0.499500       | 0.173888        | 0.188506       | 0.038599        |
| Snout length                           | 0.221800        | 0.054387         | 0.571074             | 0.240857       | 0.785650        | 0.081596       | 0.188958        |
| Mouth width                            | <b>0.428940</b> | <b>0.287830</b>  | 0.015738             | 0.270563       | 0.149392        | 0.014194       | 0.278599        |
| Eye diameter                           | <b>0.322050</b> | <b>-0.304680</b> | 0.189483             | 0.081596       | 0.384145        | 0.695834       | 0.052732        |
| Head length                            | -0.041274       | -0.079231        | 0.025348             | 0.749068       | 0.663459        | 0.188506       | 0.038599        |
| External length of the opercular organ | 0.101010        | 0.214590         | 0.029523             | 0.126449       | 0.210923        | 0.455451       | 0.469976        |
| Internal length of the opercular organ | -0.198310       | 0.162610         | 0.387252             | 0.020880       | 0.956622        | 0.165718       | 0.450161        |
| Post-opercular organ length            | -0.014224       | <b>0.348010</b>  | 0.834683             | 0.644059       | 0.355134        | 0.545714       | 0.818226        |
| Pre-dorsal distance                    | -0.021502       | -0.119360        | 0.788447             | 0.001220       | 0.000331        | 0.001220       | 0.000913        |
| Pectoral-pelvic distance               | 0.137270        | -0.130310        | 0.144045             | 0.374260       | 0.327546        | 0.188506       | 0.818226        |
| Pectoral-anal distance                 | 0.077509        | -0.128190        | 0.144045             | 0.009474       | 0.703389        | 0.971648       | 0.178261        |

|                         |                  |                  |          |          |                 |          |          |
|-------------------------|------------------|------------------|----------|----------|-----------------|----------|----------|
| Post-dorsal distance    | 0.052916         | -0.002752        | 0.025348 | 0.000949 | 0.005537        | 0.006207 | 0.250500 |
| Pre-pelvic distance     | 0.013185         | -0.084710        | 0.199839 | 0.059611 | 0.000407        | 0.036002 | 0.001449 |
| Pre-anal distance       | 0.006945         | -0.152460        | 0.018507 | 0.003992 | <b>0.000090</b> | 0.014194 | 0.002263 |
| Pre-pectoral distance   | -0.111650        | -0.145150        | 0.018507 | 0.270563 | 0.091763        | 0.004989 | 0.576741 |
| Body height             | 0.226360         | -0.095585        | 0.654721 | 0.003179 | 0.355134        | 0.644059 | 0.450161 |
| Body width              | <b>0.286560</b>  | 0.217800         | 0.029488 | 0.020880 | 0.586491        | 0.222527 | 0.019714 |
| Caudal peduncle height  | -0.221950        | <b>0.253920</b>  | 0.144045 | 0.240857 | 0.956622        | 0.644059 | 0.973808 |
| Caudal peduncle length  | 0.190230         | -0.021535        | 0.742945 | 0.270563 | 0.870378        | 0.212526 | 0.818226 |
| Dorsal height           | -0.110120        | -0.082028        | 0.296718 | 0.455451 | 0.173888        | 0.644059 | 0.308770 |
| Pelvic length           | 0.009100         | -0.227000        | 0.128380 | 0.188506 | 0.114706        | 0.337255 | 0.490520 |
| Pectoral length         | 0.103030         | -0.150660        | 0.144045 | 0.188506 | 0.301388        | 0.213524 | 0.308770 |
| Upper caudal fin lobe   | -0.024431        | -0.040548        | 0.928730 | 0.165718 | 0.019342        | 0.069896 | 0.139553 |
| Lower caudal fin lobe   | 0.066129         | -0.077297        | 0.834683 | 0.081596 | 0.000907        | 0.042783 | 0.001814 |
| Dorsal fin base width   | <b>-0.314460</b> | 0.076298         | 0.296718 | 0.593875 | 0.414472        | 0.695771 | 0.045201 |
| Pelvic fin base width   | <b>-0.280820</b> | <b>-0.259410</b> | 0.834683 | 0.145068 | 0.663459        | 0.126449 | 0.224442 |
| Pectoral fin base width | 0.153420         | 0.164030         | 0.654721 | 0.025151 | 0.253351        | 0.413677 | 0.178261 |
| Anal fin base width     | -0.217400        | 0.048781         | 0.296718 | 0.302688 | 0.479501        | 0.455451 | 0.038599 |
| Pelvic-anal distance    | 0.132390         | -0.248020        | 0.179713 | 0.109746 | 0.231447        | 0.644059 | 0.122801 |
| Anal-caudal distance    | 0.031062         | 0.092155         | 0.456057 | 0.213525 | 0.102726        | 0.109746 | 0.341025 |

| Variable                               | b. MWU tests (males) |          |          |          | c. MWU tests (males) |                 |
|--|----------------------|----------|----------|----------|----------------------|-----------------|
|  | PL1                  | IntP1    | IntP2    | IntP3    | us                   | us              |
|  | vs.                  | vs.      | vs.      | vs.      | vs.                  | vs.             |
|  | ds                   | ds       | ds       | ds       | wit                  | ds              |
|  | n=19x10              | n=5x6    | n=5x5    | n=5x8    | n=56x11              | n=28x9          |
| Standard length                        | 0.945122             | 0.855132 | 0.601509 | 0.660550 | 0.932556             | 0.607724        |
| Post-orbital distance                  | 0.000488             | 0.273323 | 0.047203 | 0.143236 | 0.030275             | 0.007932        |
| Interorbital distance                  | 0.963403             | 0.361311 | 0.601509 | 1.000000 | 0.181178             | 0.750033        |
| Head height                            | 0.027638             | 0.144128 | 0.601509 | 0.464215 | 0.722261             | 0.915425        |
| Head width                             | 0.854380             | 0.465209 | 0.117186 | 0.379776 | 0.063829             | 0.190269        |
| Snout length                           | 0.002111             | 0.010588 | 0.047203 | 0.107347 | 0.000629             | 0.000876        |
| Mouth width                            | 0.000488             | 0.006170 | 0.009024 | 0.003415 | <b>0.000002</b>      | <b>0.000016</b> |
| Eye diameter                           | 0.038948             | 0.006170 | 0.016294 | 0.379776 | 0.000431             | 0.021394        |
| Head length                            | 0.408863             | 1.000000 | 0.601509 | 0.187684 | 0.037356             | 0.777026        |
| External length of the opercular organ | 0.890518             | 0.100349 | 0.464703 | 0.379776 | 0.025470             | 0.478951        |
| Internal length of the opercular organ | 0.713571             | 0.044611 | 0.916815 | 0.040425 | 0.115465             | 0.339179        |
| Post-opercular organ length            | 0.019282             | 0.067890 | 0.347208 | 0.143236 | 0.034370             | 0.004627        |
| Pre-dorsal distance                    | 0.021782             | 0.006170 | 0.016294 | 0.008416 | 0.760621             | 0.524000        |
| Pectoral-pelvic distance               | 0.000202             | 0.067890 | 0.075801 | 0.005414 | <b>0.000172</b>      | 0.000175        |
| Pectoral-anal distance                 | 0.000955             | 0.361311 | 0.075801 | 0.057041 | <b>0.000039</b>      | 0.033673        |
| Post-dorsal distance                   | 0.312770             | 0.006170 | 0.009024 | 0.005414 | 0.051601             | 0.023478        |
| Pre-pelvic distance                    | 0.003319             | 1.000000 | 0.754023 | 1.000000 | 0.084273             | 0.051538        |
| Pre-anal distance                      | 0.183318             | 0.044611 | 0.117186 | 0.078984 | 0.326254             | 0.178566        |
| Pre-pectoral distance                  | 0.462870             | 0.044611 | 0.347208 | 0.012827 | 0.001550             | 0.202527        |
| Body height                            | 0.010186             | 0.010588 | 0.754023 | 0.057041 | <b>0.000013</b>      | 0.001127        |
| Body width                             | <b>0.000013</b>      | 0.006170 | 0.009024 | 0.003415 | 0.067552             | <b>0.000008</b> |
| Caudal peduncle height                 | 0.232885             | 0.465209 | 0.347208 | 0.143236 | 0.000521             | 0.478951        |
| Caudal peduncle length                 | <b>0.000044</b>      | 0.006170 | 0.347208 | 0.003415 | <b>0.000023</b>      | <b>0.000022</b> |
| Dorsal height                          | 0.383329             | 0.583883 | 0.174526 | 0.464215 | 0.006430             | 0.096157        |
| Pelvic length                          | 0.001123             | 0.100349 | 0.117186 | 0.379776 | 0.070135             | 0.010811        |
| Pectoral length                        | 0.000345             | 0.273323 | 0.464703 | 0.057041 | 0.905688             | 0.001276        |
| Upper caudal fin lobe                  | 0.013224             | 0.855132 | 0.347208 | 0.883618 | 0.697062             | 0.167408        |

|                         |                 |          |          |          |                 |                 |
|-------------------------|-----------------|----------|----------|----------|-----------------|-----------------|
| Lower caudal fin lobe   | <b>0.000024</b> | 0.273323 | 0.916815 | 0.040425 | 0.369687        | 0.000457        |
| Dorsal fin base width   | 0.581863        | 0.715001 | 0.464703 | 0.660550 | <b>0.000009</b> | 0.831787        |
| Pelvic fin base width   | 0.000202        | 0.006170 | 0.009024 | 0.028109 | 0.003601        | <b>0.000040</b> |
| Pectoral fin base width | 0.003319        | 0.006170 | 0.009024 | 0.040425 | 0.553584        | 0.002072        |
| Anal fin base width     | 0.183318        | 0.273323 | 0.601509 | 0.241567 | 0.006108        | 0.436103        |
| Pelvic–anal distance    | 0.581910        | 0.144128 | 0.347208 | 0.558185 | <b>0.000006</b> | 0.620181        |
| Anal–caudal distance    | 0.027638        | 0.010588 | 0.250593 | 0.057041 | 0.186768        | 0.008804        |

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**Table S14. (a)** Factor loadings for the second and third axes of a PCA performed on 31 measurements for all specimens examined (n=167). The most important values are in bold. **(b)** Results (p-values) of the MWU tests of the 31 measurements for the comparisons between Males (M) and Females (F) of three of the most upstream populations of the Luansa River and for which comparisons were possible (Table A10). **(c)** Results (p-values) of the Mann–Whitney U tests of the 31 measurements for comparisons between Males (M) and Females (F) and this for three of the four (tentative) species for which comparisons were possible (Table A10). ds = *K. sp.* ‘lua–downstream’ (= *K. maxi* sp. nov.); us’ = *K. sp.* ‘lua–upstream’ (= *K. luansaensis* sp. nov.); wit = *K. wittei*. M = Male; and F = Female. KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; and IntP2: Intermediate Plateau 2 (Figure 2). Comparisons, indeed, were not possible between M and F of the two remaining populations, i.e., from IntP1, Intermediate plateau 1, and IntP3, Intermediate plateau 3, due to significant size class differences (Table A10). Significant values ( $p \leq 0.05$  after Bonferroni correction) in bold; and highly significant values ( $p \leq 0.001$  after Bonferroni correction) in bold and underlined.

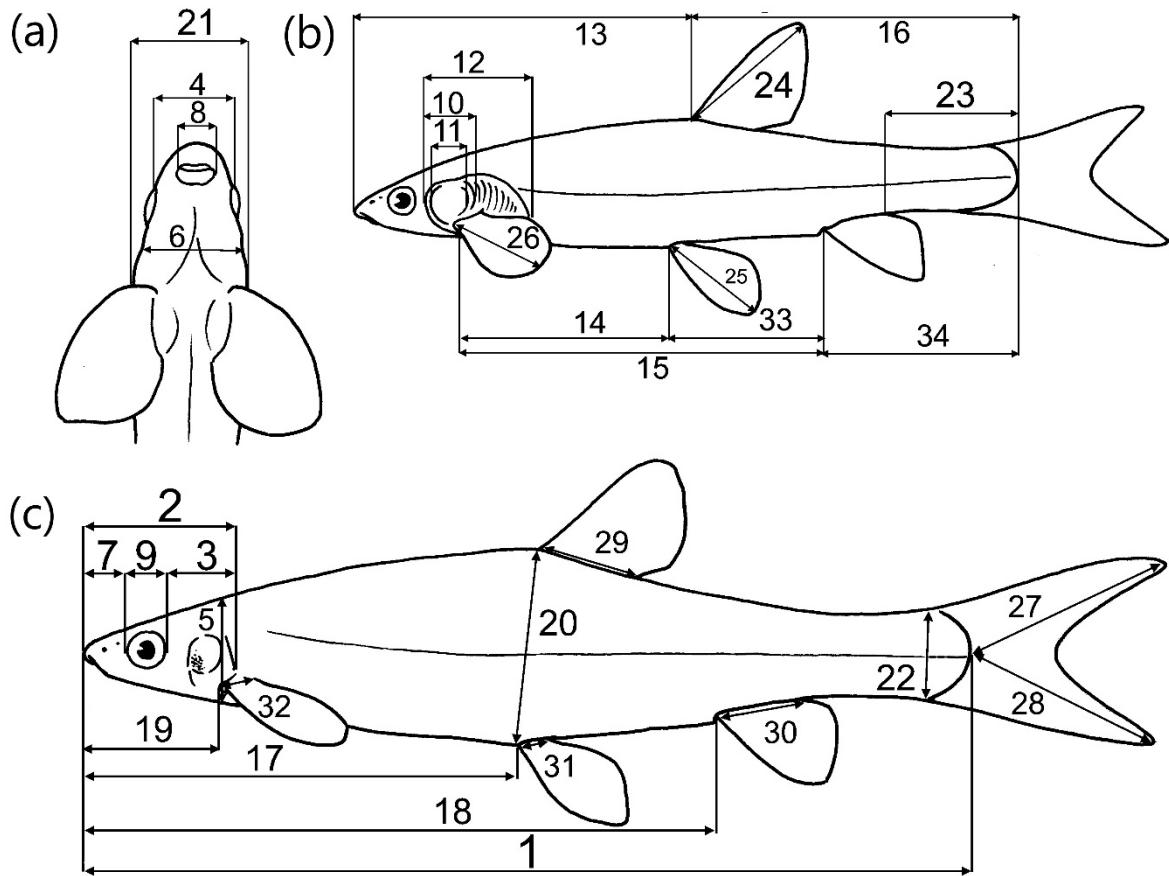
| Variable                 | a. PC Loadings   |                  | b. MWU tests (males vs. females) |                            |                               | c. MWU tests (males vs. females) |                           |                             |
|--------------------------|------------------|------------------|----------------------------------|----------------------------|-------------------------------|----------------------------------|---------------------------|-----------------------------|
|                          | PC 2             | PC 3             | KP1<br>(M vs. F)<br>n=19x13      | KP2<br>(M vs. F)<br>n=9x12 | IntP2<br>(M vs. F)<br>n=12x10 | us<br>(M vs. F)<br>n=37x39       | ds<br>(M vs. F)<br>n=10x6 | wit<br>(M vs. F)<br>n=10x10 |
|                          |                  |                  |                                  |                            |                               |                                  |                           |                             |
| Standard length          | 0.000158         | –0.043005        | 0.631500                         | 0.522432                   | 0.509652                      | 0.509299                         | 0.587594                  | 0.832689                    |
| Postorbital distance     | –0.074061        | <b>–0.283540</b> | 0.257678                         | 0.393769                   | 0.166026                      | 0.751263                         | 0.913627                  | 1.000000                    |
| Interorbital distance    | –0.204230        | –0.133760        | 0.095105                         | 0.155219                   | 0.428664                      | 0.078153                         | 0.664390                  | 0.034582                    |
| Head height              | –0.092975        | –0.097037        | 0.011965                         | 0.064641                   | 1.000000                      | 0.240260                         | 0.828263                  | 0.672655                    |
| Head width               | <b>–0.272360</b> | 0.053064         | <b>0.000061</b>                  | 0.522432                   | 0.008352                      | <b>0.000281</b>                  | 0.744882                  | 0.121336                    |
| Snout length             | <b>–0.259300</b> | 0.118800         | 0.715478                         | 0.776205                   | 0.051689                      | 0.166912                         | 0.039319                  | 1.000000                    |
| Mouth width              | –0.157600        | <b>0.427910</b>  | 0.923581                         | 0.002244                   | 0.766616                      | <b>0.000444</b>                  | 0.232824                  | 0.724771                    |
| Eye diameter             | –0.115940        | 0.153010         | 0.501925                         | 0.001075                   | 0.146768                      | <b>0.004263</b>                  | 0.328972                  | 0.324207                    |
| Head length              | –0.120260        | –0.116970        | 0.274163                         | 0.000497                   | 0.428795                      | <b>0.000025</b>                  | 0.232824                  | 0.672655                    |
| Pre–dorsal distance      | –0.056495        | –0.099924        | 0.129624                         | 0.008552                   | <b>0.000131</b>               | <b>0.000012</b>                  | <b>0.001138</b>           | 0.121215                    |
| Pectoral–pelvic distance | –0.028279        | 0.101680         | 0.139617                         | 0.434370                   | 0.355939                      | 0.815119                         | 0.128886                  | 0.438579                    |
| Pectoral–anal distance   | –0.012720        | 0.042150         | 0.409405                         | 0.355555                   | 0.947427                      | 0.799023                         | 0.278077                  | 0.832689                    |
| Post–dorsal distance     | 0.038010         | 0.034152         | 0.062757                         | 0.033007                   | 0.002420                      | <b>0.000000</b>                  | <b>0.001658</b>           | 0.204970                    |
| Pre–pelvic distance      | –0.056138        | –0.039142        | 0.022432                         | 0.477290                   | 0.006863                      | <b>0.000067</b>                  | 0.039319                  | 0.573202                    |
| Pre–anal distance        | –0.027129        | –0.046630        | 0.744319                         | 0.064641                   | 0.003005                      | <b>0.000453</b>                  | 0.913627                  | 0.121336                    |



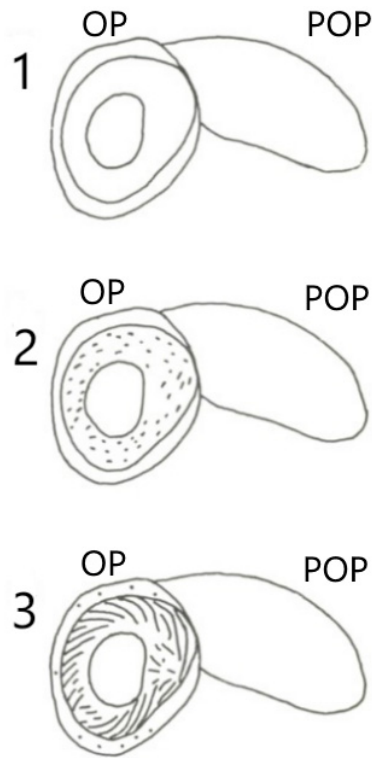
|                         |                 |                  |                 |                 |                 |                 |                 |                 |
|-------------------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Pre-pectoral distance   | -0.177540       | -0.221860        | 0.010724        | 0.001079        | 0.146882        | <u>0.000000</u> | 0.158526        | 0.048645        |
| Body height             | -0.174260       | <b>0.259670</b>  | 0.001548        | 0.004474        | 0.509652        | <b>0.001922</b> | 0.232824        | 0.169569        |
| Body width              | -0.148750       | 0.243020         | 0.046001        | 1.000000        | 0.322493        | 0.420571        | 0.913627        | 0.204970        |
| Caudal peduncle height  | 0.152270        | -0.123960        | 0.501925        | 0.886974        | 0.391338        | <b>0.008048</b> | 0.103743        | 0.011244        |
| Caudal peduncle length  | 0.021391        | 0.090613         | 0.027354        | 0.255510        | 0.146882        | <u>0.000010</u> | 0.328972        | 0.159026        |
| Dorsal height           | <b>0.344660</b> | 0.042509         | <u>0.000003</u> | <b>0.000124</b> | <b>0.000100</b> | <u>0.000000</u> | <b>0.001138</b> | <b>0.000251</b> |
| Pelvic length           | 0.160230        | -0.009847        | <u>0.000004</u> | 0.039310        | 0.005616        | <u>0.000000</u> | 0.022741        | 0.048645        |
| Pectoral length         | -0.170140       | -0.028178        | 0.893171        | <b>0.000497</b> | 0.006863        | <u>0.000005</u> | 0.158526        | 0.180917        |
| Upper caudal fin lobe   | 0.119810        | -0.017947        | <b>0.000084</b> | 0.434370        | 0.644393        | <b>0.000401</b> | 0.128886        | 0.069643        |
| Lower caudal fin lobe   | <b>0.371430</b> | 0.035504         | <u>0.000002</u> | <b>0.000166</b> | 0.008352        | <u>0.000000</u> | 0.232824        | 0.061185        |
| Dorsal fin base width   | <b>0.263500</b> | -0.166340        | 0.000786        | 0.915051        | 0.075023        | <u>0.000002</u> | 0.232824        | <b>0.001531</b> |
| Pelvic fin base width   | 0.067492        | <b>-0.309390</b> | 0.773523        | 0.088082        | 0.428795        | 0.904870        | 0.103743        | 0.778196        |
| Pectoral fin base width | <b>0.376350</b> | <b>0.519770</b>  | <u>0.000003</u> | <b>0.000220</b> | <b>0.000076</b> | <u>0.000000</u> | <b>0.001138</b> | <b>0.000108</b> |
| Anal fin base width     | <b>0.291630</b> | -0.167820        | 0.003333        | 0.669816        | 0.575049        | <u>0.000131</u> | 0.158526        | 0.324207        |
| Pelvic-anal distance    | -0.011927       | 0.032065         | 0.120195        | 0.200826        | 0.791971        | 0.291509        | 0.664390        | 0.231267        |
| Anal-caudal distance    | 0.079481        | 0.006358         | 0.2268034       | 0.4343704       | 0.0249689       | <u>0.000001</u> | 0.065197        | 0.204970        |

**Table S15.** Tubercle development on the body in function of the three development stage of the tubercles/lamellae in the opercular organ (T/L–OP). VPH: Ventral Part of Head, i.e. below mid–eye level; DPH: Dorsal Part of Head, i.e. above mid–eye level; DPT: Dorsal Part of Trunk; and VPT: Ventral Part of Trunk. n: number of specimens. –: absence; +: presence. KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; IntP3: Intermediate plateau 3 (Figure 2).

| Species                        | Population | T/L–OP stage | N° | VPH | DPH | DPT | VPT |
|--------------------------------|------------|--------------|----|-----|-----|-----|-----|
| <i>K. sp. 'lua-upstream'</i>   | KP1L       | 1            | 12 | –   | –   | –   | –   |
|                                |            | 1            | 1  | +   | +   | –   | –   |
|                                |            | 2            | 2  | +   | +   | –   | –   |
|                                |            | 3            | 2  | +   | +   | –   | –   |
|                                |            | 3            | 2  | +   | +   | +   | +   |
|                                | KP2M       | 1            | 14 | –   | –   | –   | –   |
|                                |            | 2            | 1  | +   | –   | –   | –   |
|                                | IntP1      | 1            | 5  | +   | +   | –   | –   |
|                                |            | 2            | 2  | +   | +   | +   | +   |
|                                |            | 3            | 3  | +   | +   | +   | +   |
|                                | IntP2      | 1            | 2  | +   | +   | –   | –   |
|                                |            | 1            | 8  | –   | –   | –   | –   |
|                                |            | 2            | 1  | –   | –   | –   | –   |
|                                |            | 2            | 3  | +   | –   | –   | –   |
|                                |            | 2            | 1  | +   | –   | –   | +   |
|                                | IntP3      | 3            | 1  | +   | +   | +   | –   |
|                                |            | 3            | 1  | +   | +   | +   | +   |
|                                |            | 1            | 4  | +   | –   | –   | –   |
|                                |            | 2            | 1  | +   | –   | –   | –   |
|                                |            | 2            | 1  | –   | –   | –   | –   |
| <i>K. sp. 'lua-downstream'</i> |            | 1            | 1  | –   | –   | –   | –   |
|                                |            | 1            | 1  | +   | –   | –   | –   |
|                                |            | 2            | 3  | –   | –   | –   | –   |
|                                |            | 2            | 1  | +   | –   | –   | –   |
|                                |            | 3            | 2  | –   | –   | –   | –   |
|                                |            | 3            | 1  | +   | –   | –   | –   |
|                                |            | 3            | 2  | +   | +   | +   | –   |
|                                |            | 3            | 2  | +   | +   | +   | –   |
| <i>K. stappersii</i>           |            | 1            | 10 | –   | –   | –   | –   |
|                                |            | 3            | 1  | +   | +   | +   | –   |
|                                |            | 2            | 1  | +   | +   | +   | –   |
|                                |            | 2            | 4  | –   | –   | –   | –   |
| <i>K. wittei</i>               |            | 1            | 45 | –   | –   | –   | –   |



**Figure S1.** Schematic illustration of the measurements taken on the *Kneria* specimens examined: (a) ventral view of head; (b) lateral view of male specimen to illustrate, especially, the opercular and post opercular organ related measurements as well as some body measurements taken on both sexes; and (c) lateral view of female specimen to illustrate the head and additional body measurements taken on both sexes as well. 1. Standard length ( $L_s$ ); 2. Head length ( $L_H$ ); 3. Post-orbital distance; 4. Interorbital distance; 5. Head height; 6. Head width; 7. Snout length; 8. Mouth width; 9. Eye diameter; 10. External length of the opercular organ; 11. Internal length of the opercular organ; 12. Opercular organ length; 13. Pre-dorsal distance; 14. Pectoral-pelvic distance; 15. Pectoral-anal distance; 16. Post-dorsal distance; 17. Pre-pelvic distance; 18. Pre-anal distance; 19. Pre-pectoral distance; 20. Body height; 21. Body width; 22. Caudal-peduncle height; 23. Caudal-peduncle length; 24. Dorsal height; 25. Pelvic length; 26. Pectoral length; 27. Length of longest caudal fin ray of upper lobe; 28. Length of longest caudal fin ray of lower lobe; 29. Dorsal fin base width; 30. Anal fin base width; 31. Pelvic fin base width; 32. Pectoral fin base width; 33. Pelvic-anal distance; and 34. Anal-caudal distance.

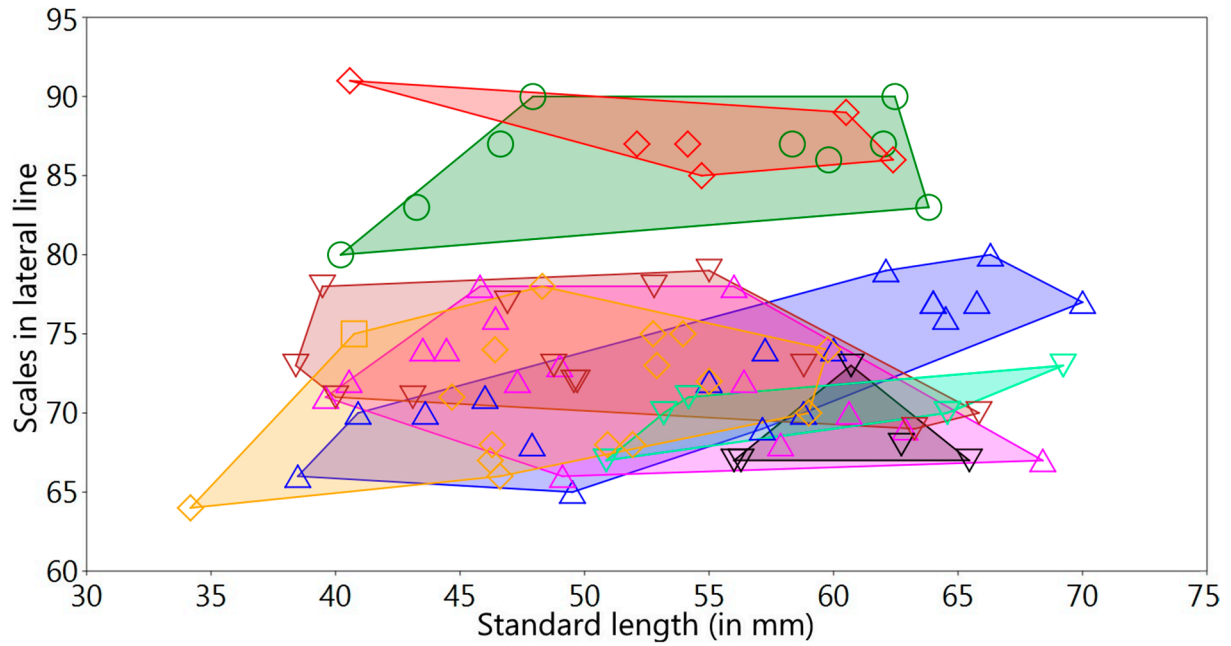


**Figure S2.** Schematic illustration of the three different ways in which the tubercular/lamellar structures can present themselves in the OP organ of male *Kneria* specimens (from Peters [55]: 412–415, Fig. 8) and also observed in the males of *K. stappersii*, *K. wittei* and *Kneria* spp. from the Luansa River. OP organ **(1)** without lamellae, **(2)** with tubercles towards its outer region, and **(3)** with lamellae towards its outer region. OP, Opercular organ; and POP, post-opercular organ.



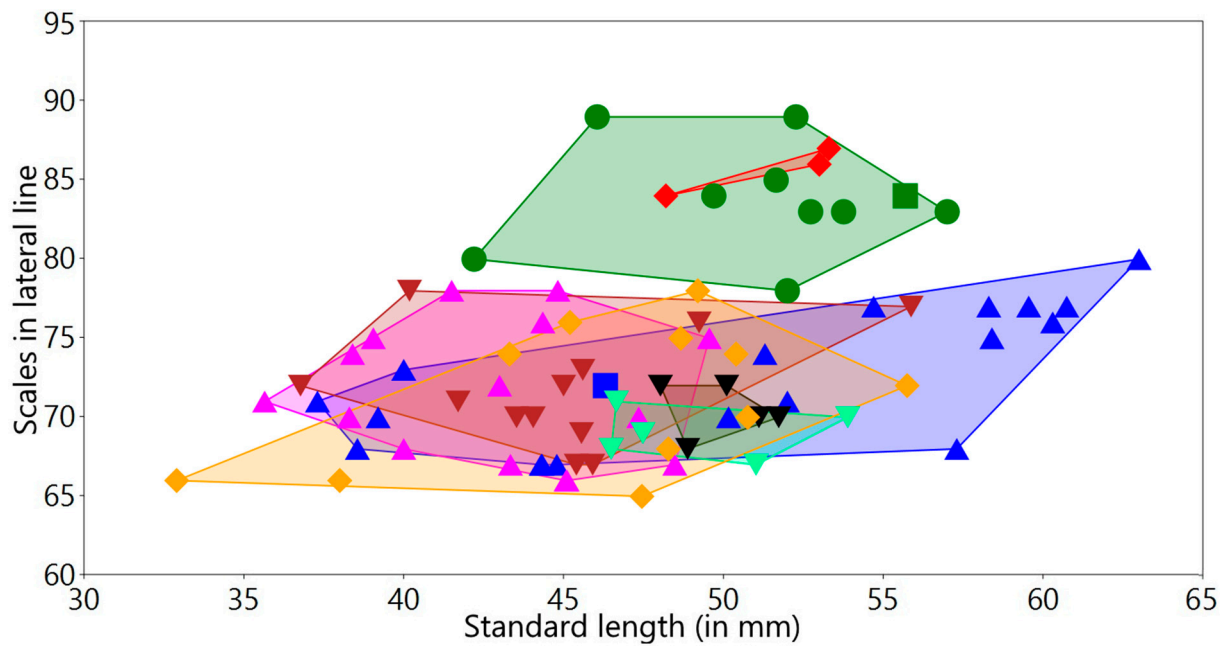


**Figure S3.** Life photographs of: **(a1)** *Kneria* sp. 'lua-upstream' (= *K. sp. luansaensis* sp. nov.) ♂ RMCA 2018-020-P-0082-0086; DNA tag 97, 44.3mm *L<sub>s</sub>*, 21 Sep 2017: L-DS; & **(a2)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♀ (RMCA 2018-020-P-0077-0081; DNA tag 88, 43.6mm *L<sub>s</sub>*), 21 Sep 2017: L-DS; **(b1)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♂ RMCA 2018-020-P-0052-0056; DNA tag 69, 51.1mm *L<sub>s</sub>*, 20 Sep 2017: L-DS; **(b2)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♂ RMCA 2018-020-P-0052-0056; DNA tag 70, 50.1mm *L<sub>s</sub>*, 20 Sep 2017: L-DS; and **(b3)** ♀ RMCA 2018-020-P-0047-0051; DNA tag 65, 65.5mm *L<sub>s</sub>*, 20 Sep 2017: L-DS; **(c1)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♂ RMCA 2018-020-P-0037-0041; DNA tag 48, 46.7mm *L<sub>s</sub>*, 20 Sep 2017: L-DS; **(c2)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♀ RMCA 2018-020-P-0042-0046; DNA tag 50, 69.2mm *L<sub>s</sub>*, 20 Sep 2017: L-DS; and **(c3)** *K. sp. 'lua-upstream'* (= *K. sp. luansaensis* sp. nov.) ♀ RMCA 2018-020-P-0042-0046; DNA tag 51, 53.2mm *L<sub>s</sub>*, 20 Sep 2017: L-DS. The red/orange and pink colour of the fins is an artefact of a hand keeping the specimen in place.



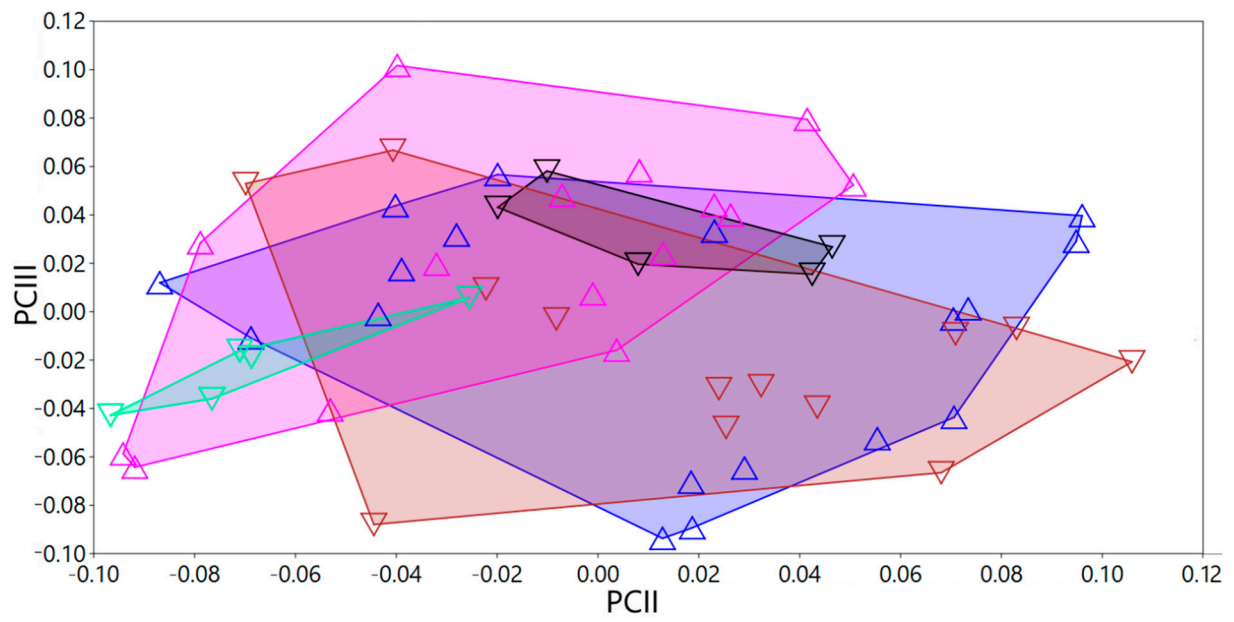
**Figure S4.** Scatterplot of the number of lateral line scales against the standard length (in mm) for all examined females of *Kneria* spp. (n=87). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.)  $\triangle$ , KP1L;  $\triangle$ , KP2M;  $\nabla$ , IntP1;  $\nabla$ , IntP2;  $\nabla$ , IntP3. *Kneria* sp. 'lua-downstream' (= *K. maxi* sp. nov.):  $\circ$ , below the last Sanshifolo Falls. *Kneria wittei*:  $\square$ , holotype;  $\diamond$ , specimens. *K. stappersii*:  $\diamond$ , specimens. All Luansa populations, from up- to downstream: KP1L: Luansa River on the Kundelungu Plateau, loc 1; KP2M: Milembwe River on the Kundelungu Plateau, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; and IntP3: Intermediate plateau 3; ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); primary types (square).



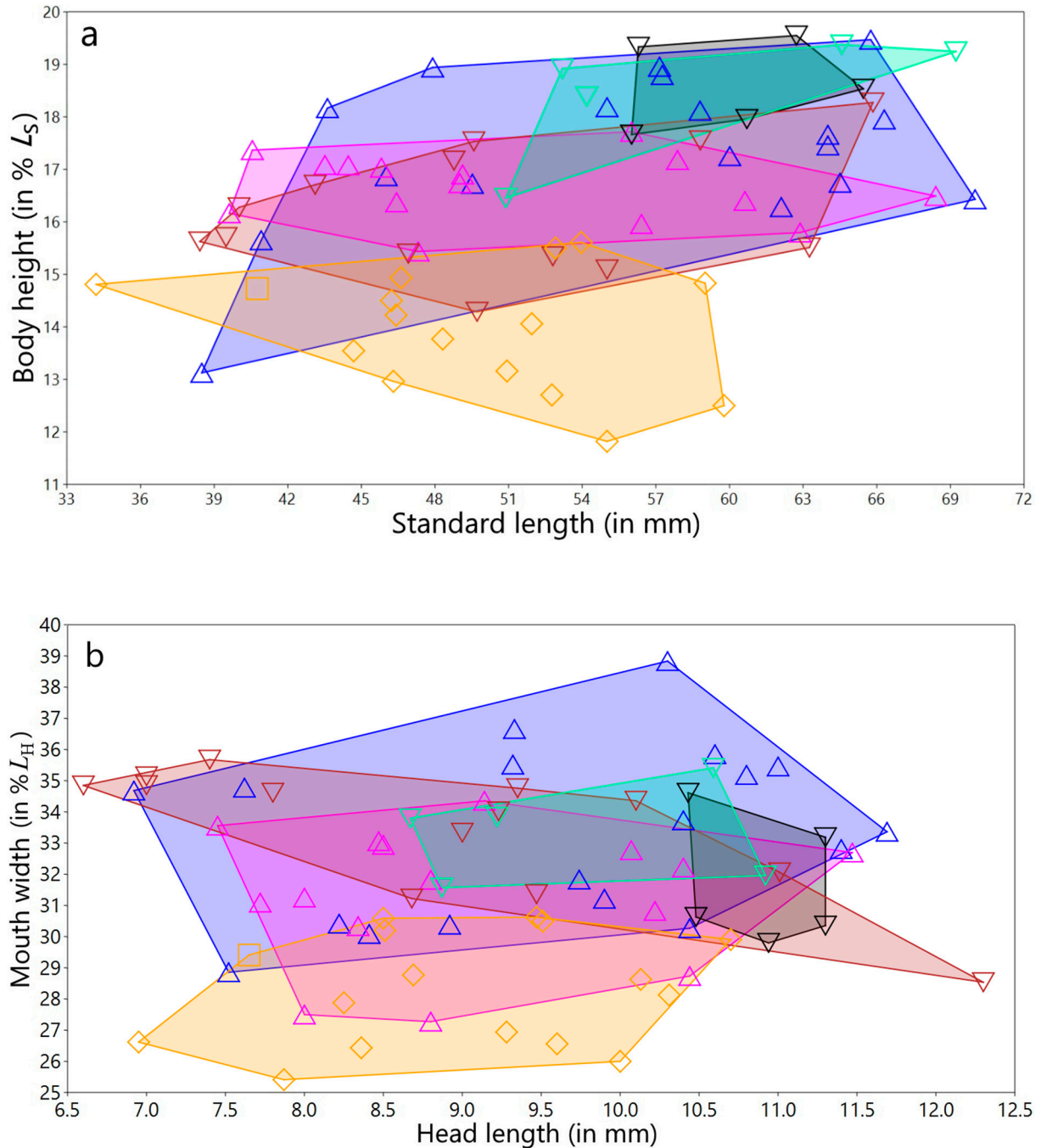


**Figure S5.** Scatterplot of the number of lamellae in the post opercular (POP) organ against the standard length ( $L_s$ ) for all examined males of *Kneria* spp. ( $n=80$ ). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.): ■, holotype, ▲, specimens from KP1L; ▲, KP2M; ▼, IntP1; ▼, IntP2; and ▼, IntP3; *K. sp.* 'lua-downstream' (= *K. maxi* sp. nov.) from below the last Sanshifolo Falls: ■, holotype; ●, specimens; *K. wittei*: ◆, specimens; and *K. stappersii*: ◆, specimens. All Luansa populations, from up- to downstream: P1L: Plateau one on the Luansa River, P2M: Plateau two on the Milembwe River, IntP1: Intermediate plateau 1, IntP2: Intermediate plateau 2, IntP3: Intermediate plateau 3, ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); primary types (square).

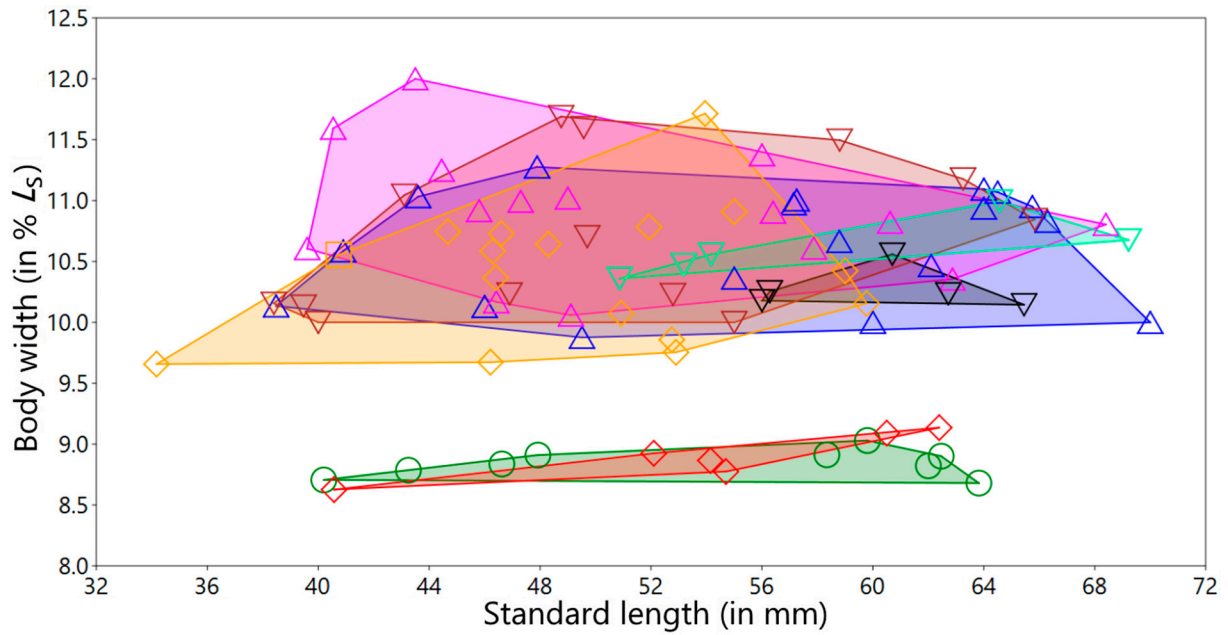




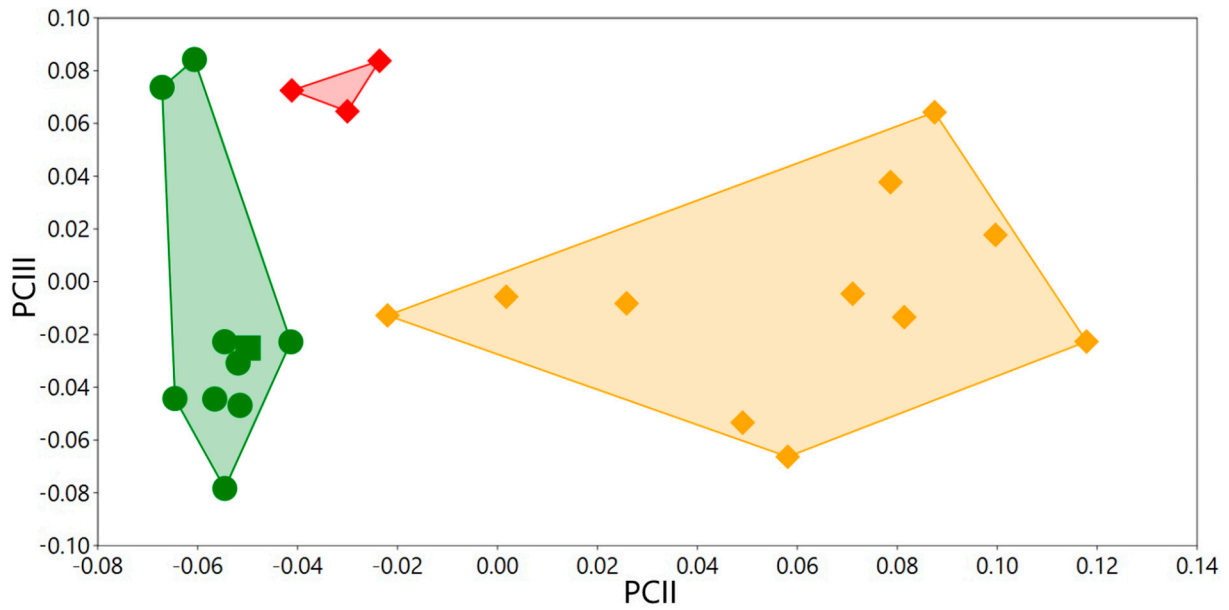
**Figure S6.** Scatterplot of PCII against PCIII of a PCA on 31 log-transformed measurements performed on all females of *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.) (n=56). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.):  $\triangle$ , KP1L;  $\triangle$ , KP2M;  $\nabla$ , IntP1;  $\nabla$ , IntP2; and  $\nabla$ , IntP3.



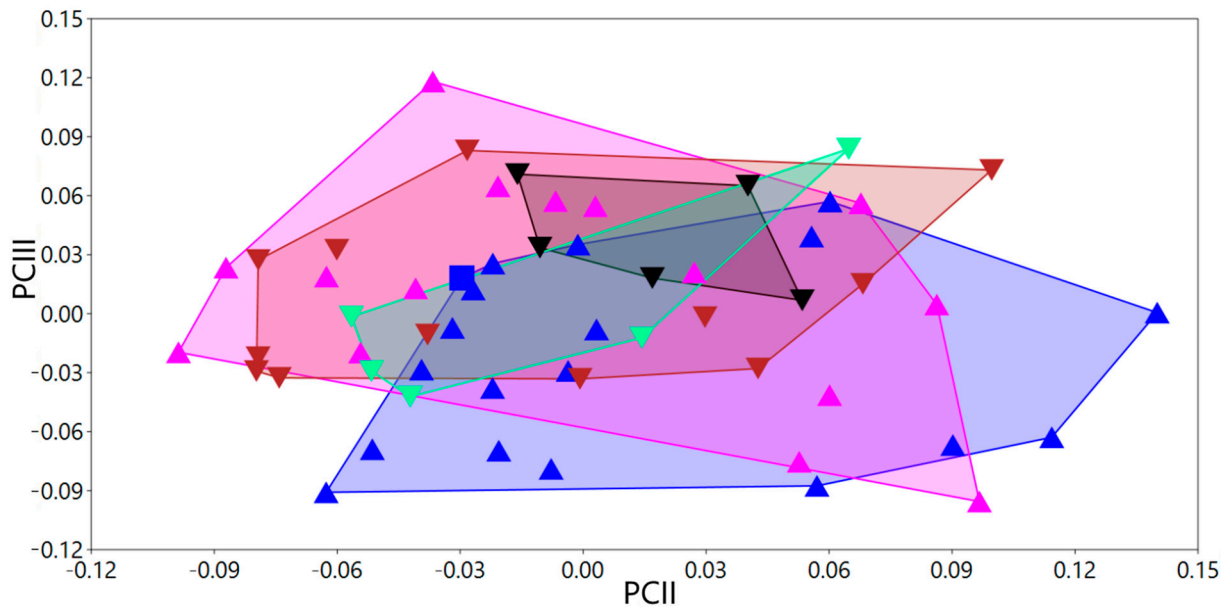
**Figure S7.** Scatterplot of: **(a)** body height (in %  $L_S$ ) against standard length ( $L_S$ ) in mm; and **(b)** mouth width (in %  $L_H$ ) against head length ( $L_H$ ) in mm for all females ( $n=72$ ). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.):  $\triangle$ , KP1L;  $\triangle$ , KP2M;  $\nabla$ , IntP1;  $\nabla$ , IntP2;  $\nabla$ , IntP3. *K. wittei*:  $\square$ , holotype;  $\diamond$ , non-type specimens. All Luansa populations, from up- to downstream: KP1L: Luansa River on the Kundelungu Plateau, loc 1; KP2M: Milembwe River on the Kundelungu Plateau, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; and IntP3: Intermediate plateau 3. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); primary types (square).



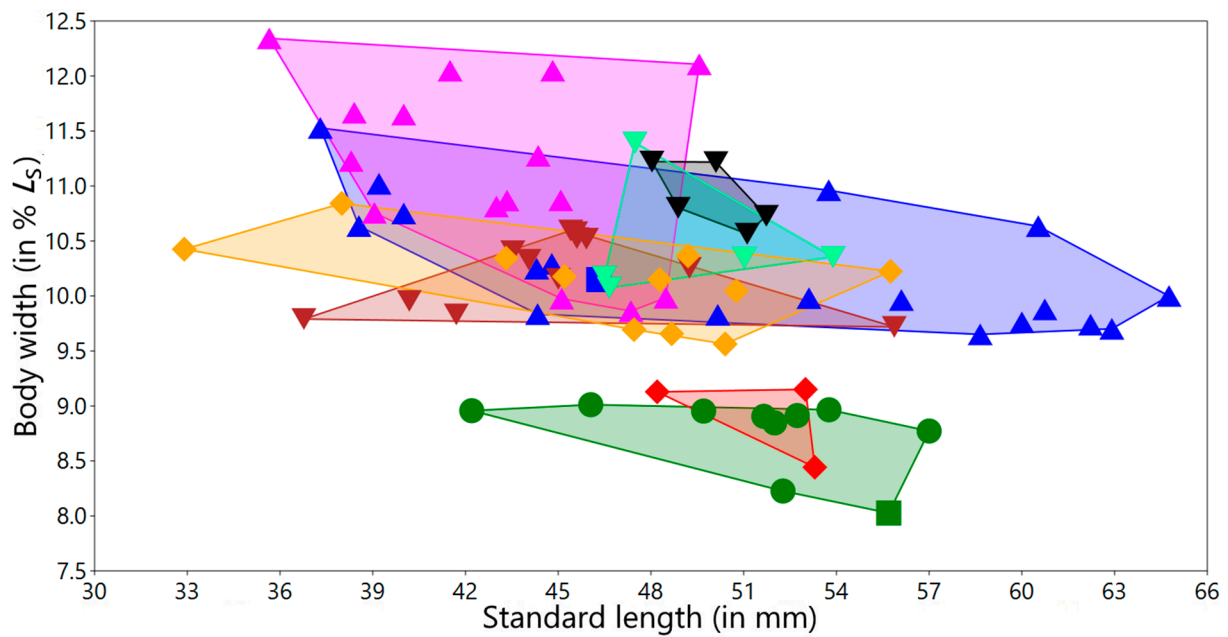
**Figure S8.** Scatterplot of body width (in %  $L_s$ ), against standard length ( $L_s$ ), in mm, for all females ( $n=87$ ). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.):  $\triangle$ , KP1L;  $\triangle$ , KP2M;  $\nabla$ , IntP1;  $\nabla$ , IntP2;  $\nabla$ , IntP3. *K. sp.* 'lua-downstream' (= *K. maxi* sp. nov.):  $\bigcirc$ , below the last Sanshifolo Falls. *K. wittei*:  $\square$ , holotype;  $\diamond$ , non-type specimens. *K. stappersii*:  $\diamond$ , non-type specimens. All Luansa populations, from up- to downstream: KP1L: Luansa River on the Kundelungu Plateau, loc 1; KP2M: Milembwe River on the Kundelungu Plateau, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; and IntP3: Intermediate plateau 3; ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); primary types (square).



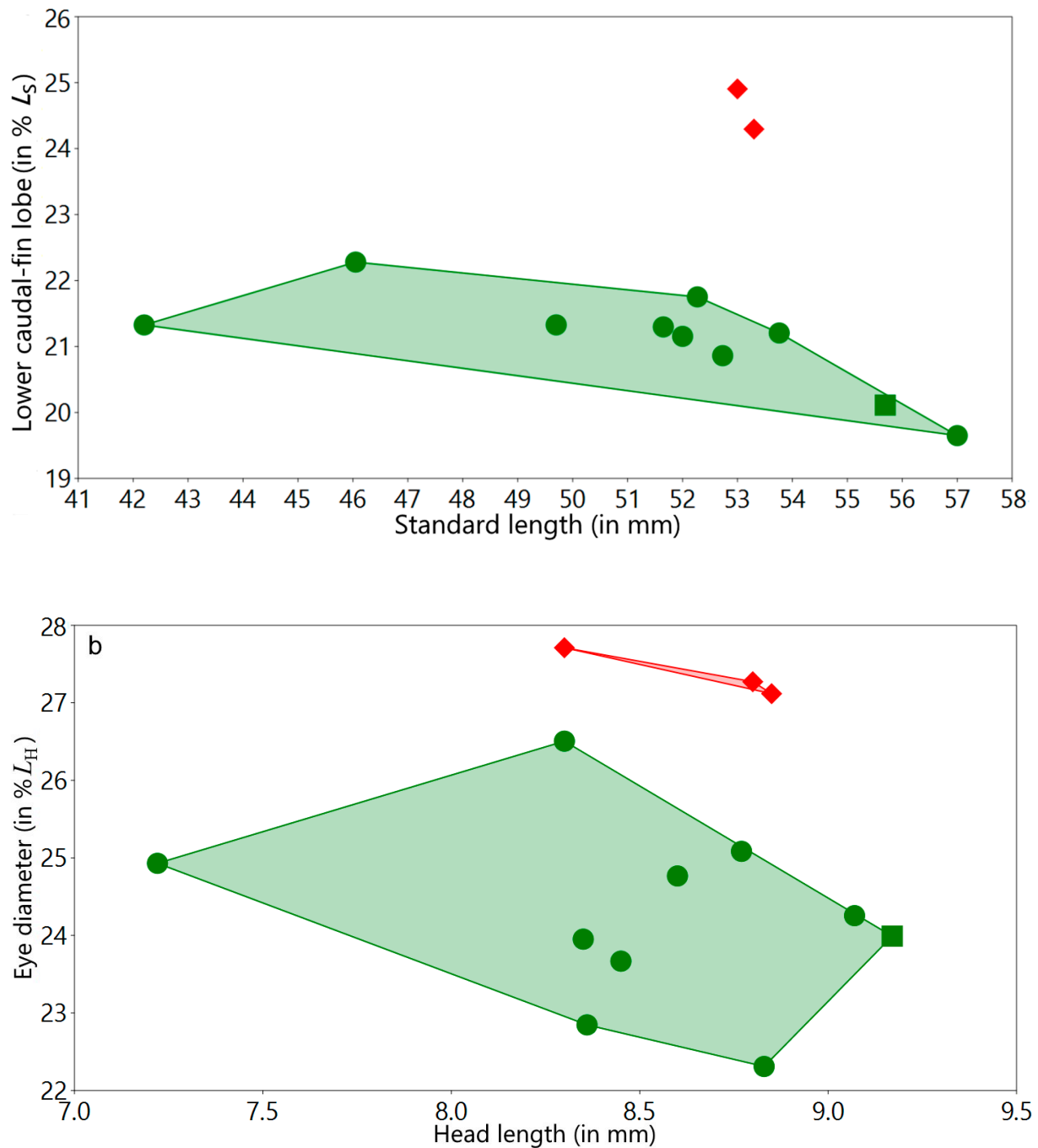
**Figure S9.** Scatterplot of PC II against PC III for a principal component analysis on 34 log-transformed measurements of males of *Kneria* spp. (n=24). *Kneria* sp. 'lua-downstream' (= *K. maxi* sp. nov.) from below the last Sanshifolo Falls: ■, holotype; ●, specimens. *Kneria wittei*, ◆ specimens; *K. stappersii*: ◆, specimens. ds: luansa downstream. Primary types (square).



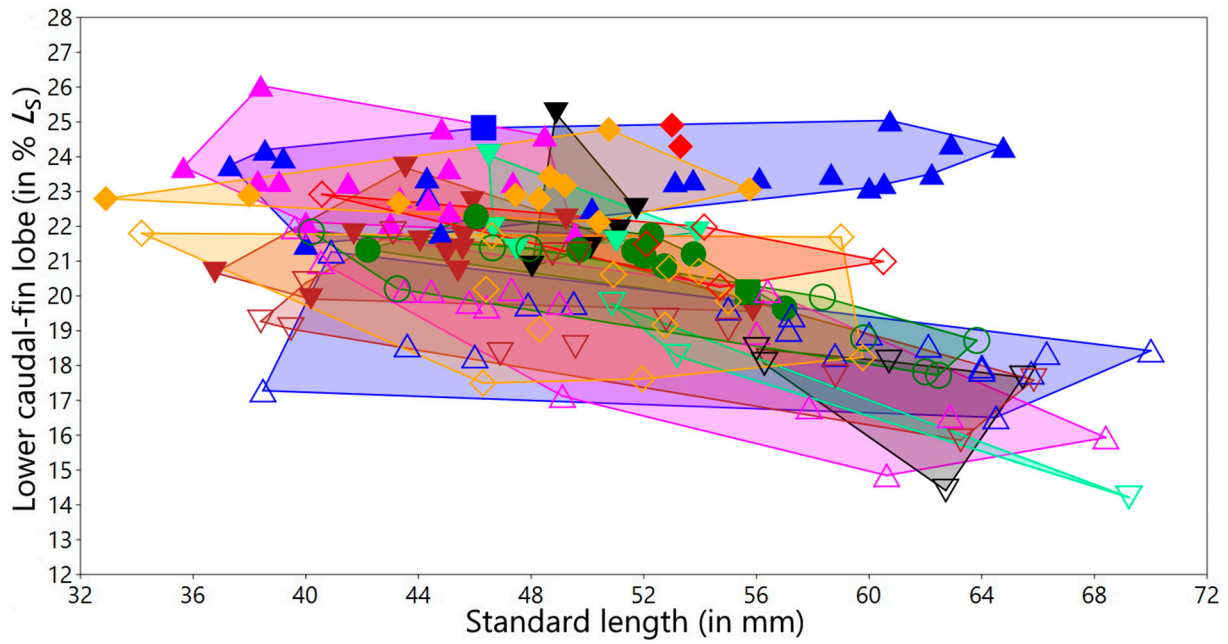
**Figure S10.** Scatterplot of PCII against PCIII of a PCA on 34 log-transformed measurements: performed on males of *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.) (n=56). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.) from: ■, holotype, ▲, specimens from KP1L; ▲, KP2M; ▼, IntP1; ▼, IntP2; and ▼, IntP3 P1L: Plateau one on the Luansa River, P2M: Plateau two on the Milembwe River, IntP1: Intermediate plateau 1, IntP2: Intermediate plateau 2, IntP3: Intermediate plateau 3, ds: downstream, lua: lansa, us: upstream. Primary types (square).



**Figure S11.** Scatterplot of: body width (in %  $L_s$ ) against standard length ( $L_s$ ) in mm for all males only ( $n=80$ ). *Kneria* sp. 'lua-upstream' (= *K. luansaensis* sp. nov.): ■, holotype, ▲, specimens from KP1L; ▲, KP2M; ▼, IntP1; ▼, IntP2; and ▼, IntP3; *K. sp. 'lua-downstream'* (= *K. maxi* sp. nov.) from below the last Sanshifolo Falls: ■, holotype; ●, specimens; *K. wittei*: ◆, specimens; and *K. stappersii*: ◆, specimens. All Luansa populations, from up- to downstream: P1L: Plateau one on the Luansa River, P2M: Plateau two on the Milembwe River, IntP1: Intermediate plateau 1, IntP2: Intermediate plateau 2, IntP3: Intermediate plateau 3, ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); primary types (square).



**Figure S12.** Scatterplot of: **(a)** Lower caudal fin lobe (in %  $L_S$ ) against standard length ( $L_S$ ) in mm; and **(b)** Eye diameter (in %  $L_H$ ) against head length ( $L_H$ ) in mm for males only for *K. sp. 'lua-downstream'* (= *K. maxi* sp. nov.) and *K. stappersii* (n=13). *K. sp. 'lua-downstream'* (= *K. maxi* sp. nov.) from below the last Sanshifolo Falls: ■, holotype; ● specimens; *K. stappersii*: ♦, specimens. ds: downstream. Primary types (square).



**Figure S13.** Scatterplot of lower caudal fin lobe (in % standard length) against standard length ( $L_s$ ) in mm for all *Kneria* spp. ( $n=167$ ). *Kneria* sp. 'lua-upstream' (*K. luansaensis* sp. nov.): ■, holotype (male), ▲, males, and △, females from KP1L; ▲, males, and △, females from KP2M; ▼, males, and ▽, females from IntP1; ▼, males, and ▽, females from IntP2; and ▼, males and ▽, females from IntP3. *Kneria* sp. 'lua-downstream' (= *K. maxi* sp. nov.): ■, holotype (male); ●, males, and ○, females from below the last Sanshifolo falls. *Kneria wittei*: □, holotype (female); ◆, male and ◇, females. *Kneria stappersii*: ◆, males, and ◇, females. All Luansa populations, from up- to downstream: KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: Intermediate plateau 1; IntP2: Intermediate plateau 2; and IntP3: Intermediate plateau 3; ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); primary types (square).





**Figure S14.** Habitat for *K. luansaensis* sp. nov., stagnant or slow moving water in a pool situated on the Milembwe River, a left-bank tributary of the Luansa River upstream of Kasompola Falls (Luansa) on the KP, upstream of Kabyashya Village (10°18'15.9"S 28°03'30.5"E); Alt. 1389m a.s.l., 21 Sep 2017.