# Supplementary information

## for

## Application of quantitative metabolomics for taxonomic differentiation of birds

by

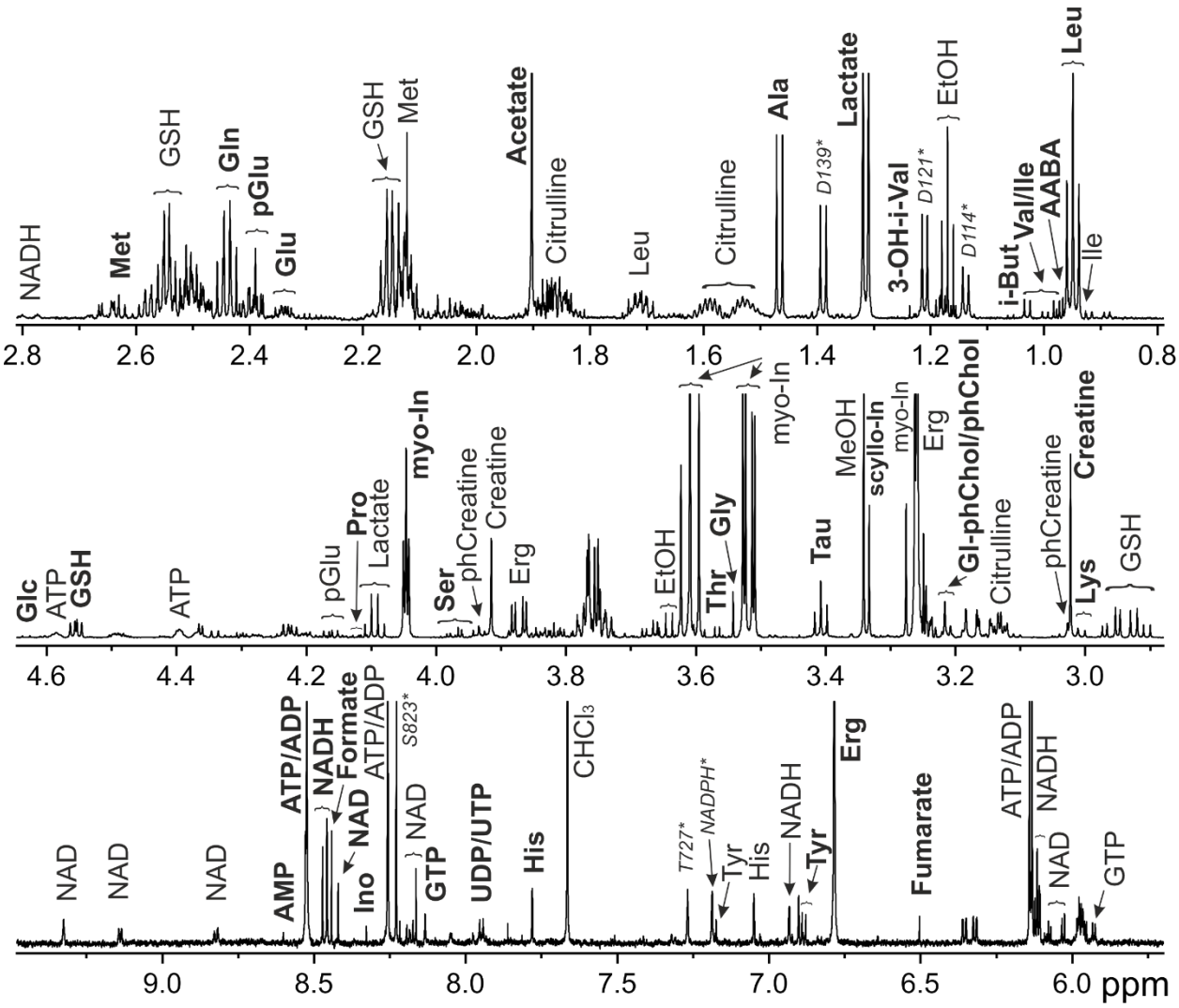
Ekaterina A. Zelentsova[1], Lyudmila V. Yanshole[1], Yuri P. Tsentalovich[1], Kirill A. Sharshov[2], and Vadim. V. Yanshole[1,*]

[1] *Laboratory of Proteomics and Metabolomics, International Tomography Center SB RAS, Institutskaya 3a, Novosibirsk, 630090, Russia*

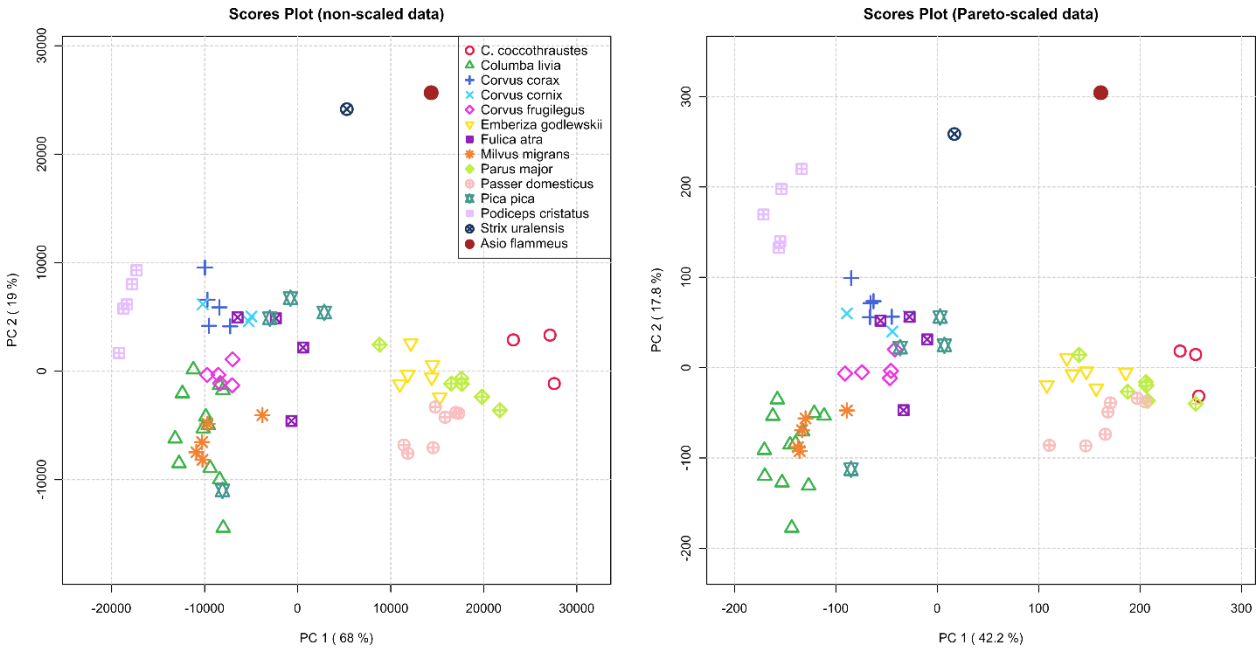
[2] *Laboratory of Molecular Epidemiology and Biodiversity of Viruses, Federal Research Center of Fundamental and Translational Medicine, Timakova str. 2, Novosibirsk, 630117, Russia*

[*] *Corresponding author. E-mail address: vadim.yanshole@tomo.nsc.ru*

Contents:




**Supplementary Figure S1. Representative $^{1}$H NMR spectrum of bird lens metabolome.** Obtained for *P. cristatus* lens. The signals used for metabolite quantification are shown in bold. Abbreviations: 3-OH-i-Val – 3-hydroxyisovalerate, AABA – 2-aminobutyrate, ADP - adenosine diphosphate, AMP - adenosine monophosphate, ATP – adenosine triphosphate, CHCl3 – chloroform, Erg – ergothioneine, EtOH – ethanol, Glc – glucose, Gl-phChol – glycero-3-phosphocholine, GSH – glutathione reduced, GTP - guanosine triphosphate, i-But – isobutyrate, Ino – inosine, MeOH – methanol, myo-In – myo-inositol, NAD - nicotinamide adenine dinucleotide, NADH – nicotinamide adenine dinucleotide reduced, NADPH - nicotinamide adenine dinucleotide phosphate reduced, pGlu – pyroglutamate, phChol – O-phosphocholine, phCreatine – phosphocreatine, scyllo-In – scyllo-inositol, Tau – taurine, UDP – uridine diphosphate, UTP – uridine triphosphate. For amino acids, standard tree letter code is used.

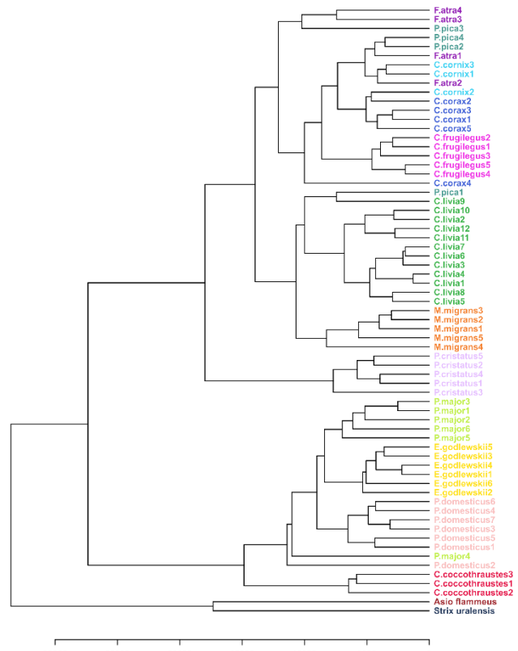


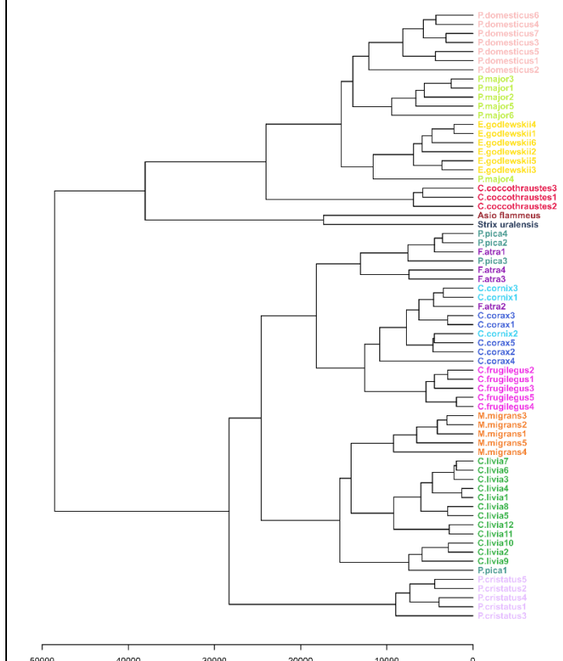

**Supplementary Figure S2. PCA scores plots for non-scaled (left panel) and Pareto-scaled (right panel) data.**

**Non-scaled data**

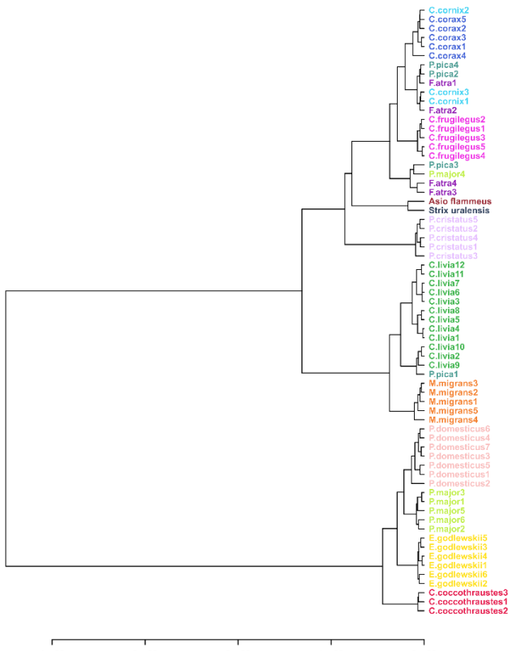
Clustering algorithm

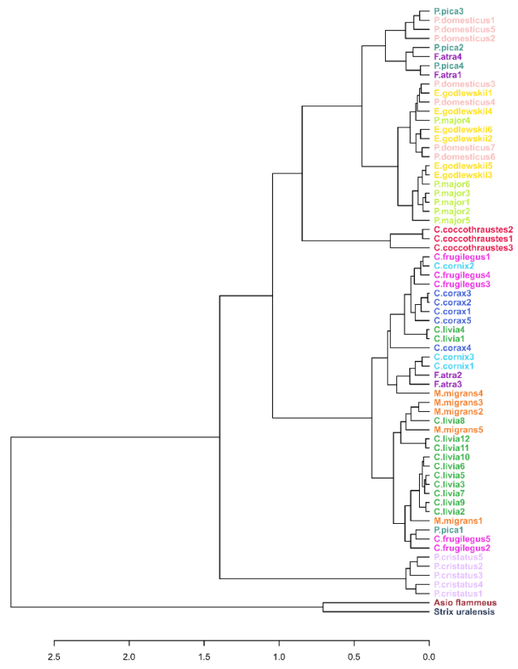
| | Average linkage | Complete linkage | Single linkage | Ward's linkage |
|---|---|---|---|---|
| Euclidean distance | NEA | NEC | NES | NEW |
| Pearson's correlation | NPA | NPC | NPS | NPW |
| Spearman's rank correlation | NSA | NSC | NSS | NSW |

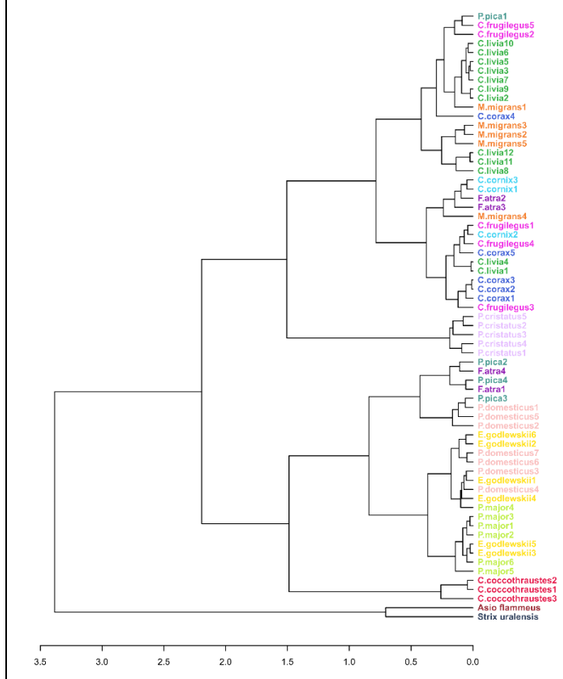


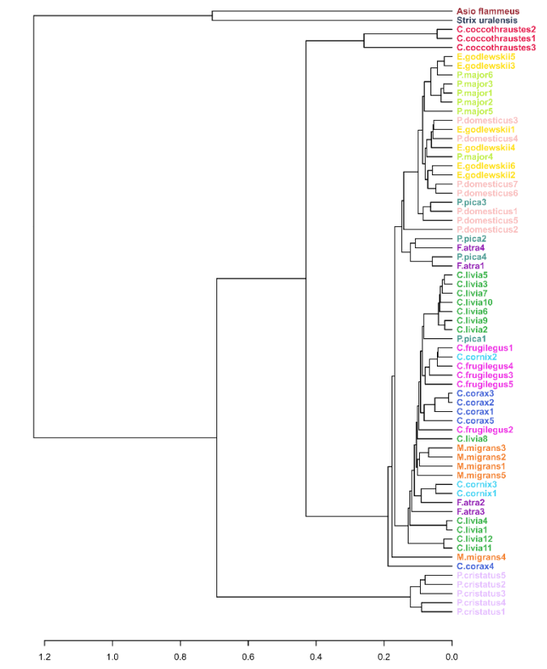



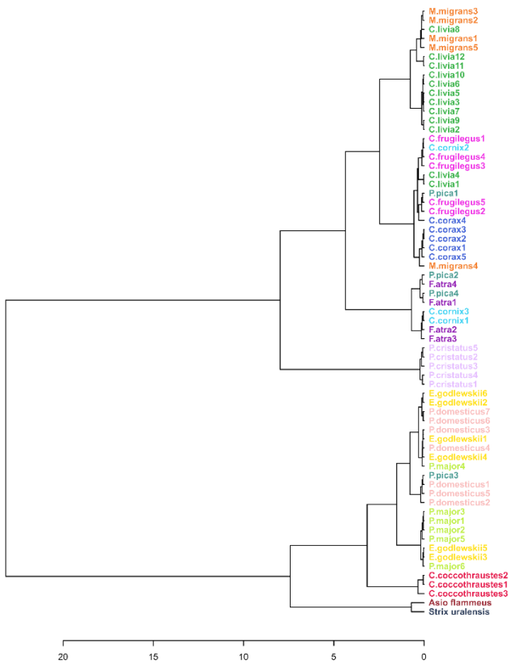


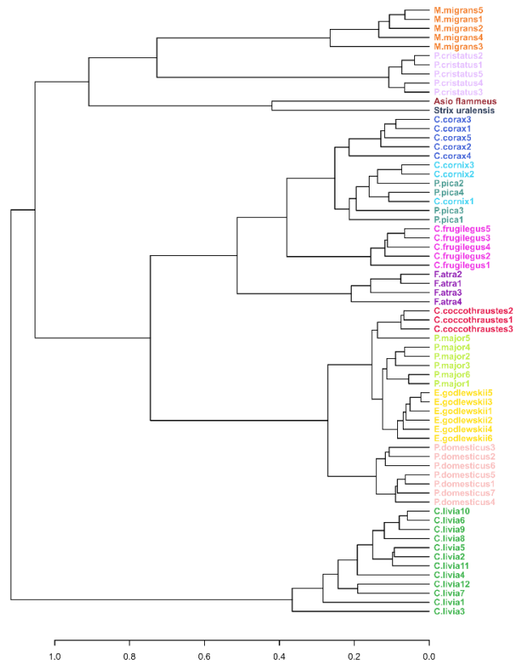


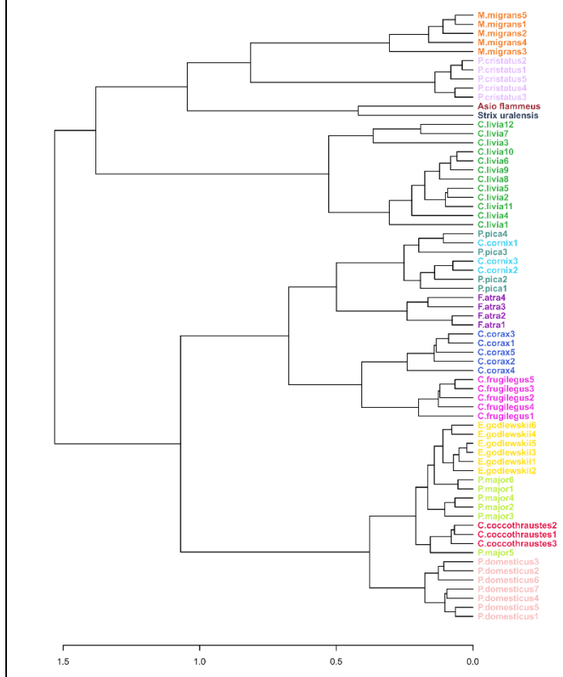



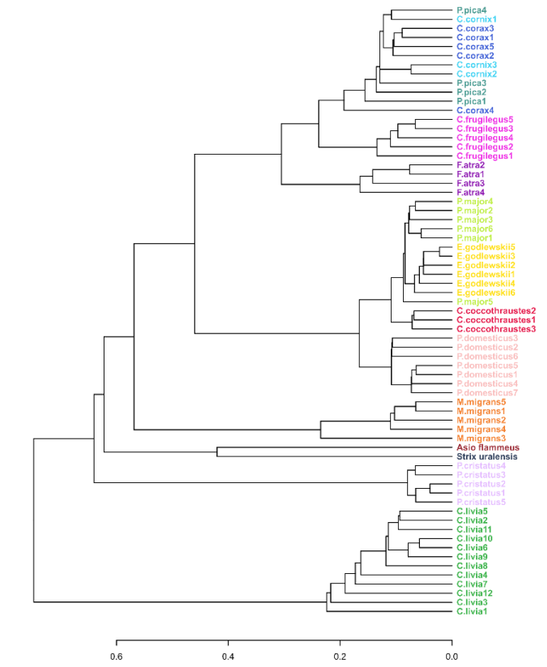



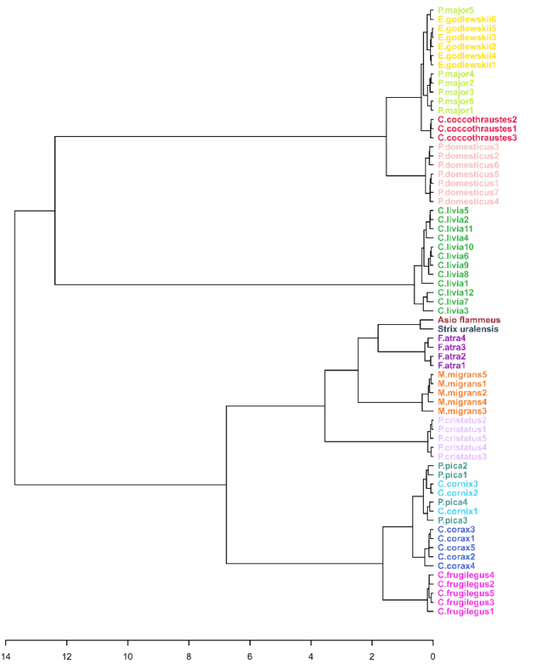



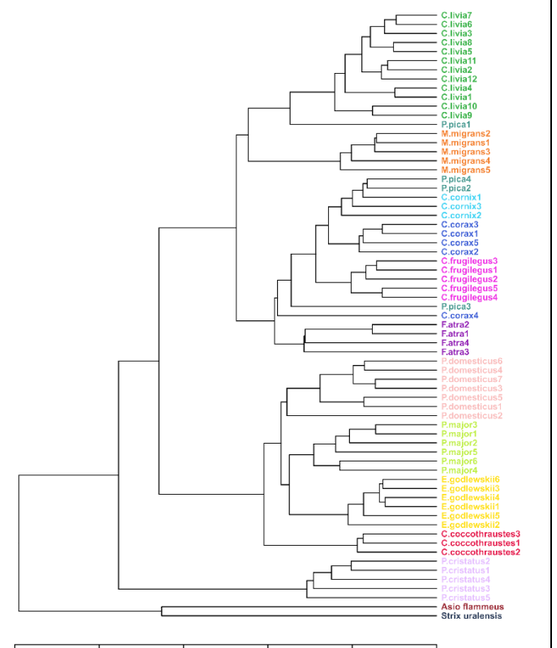



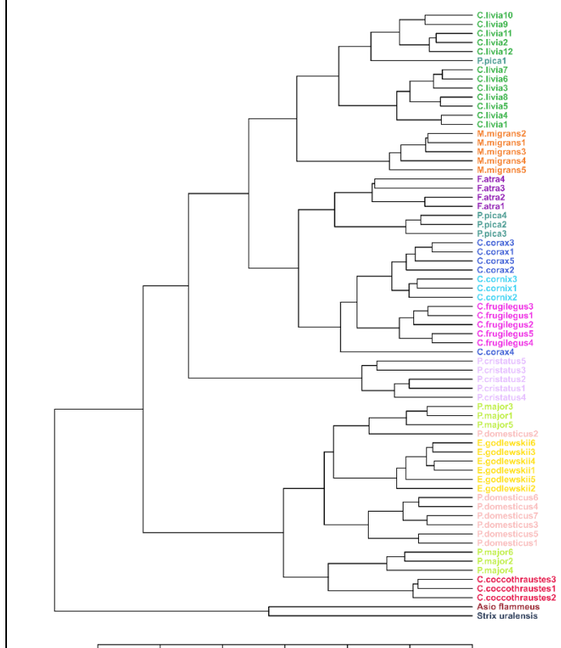



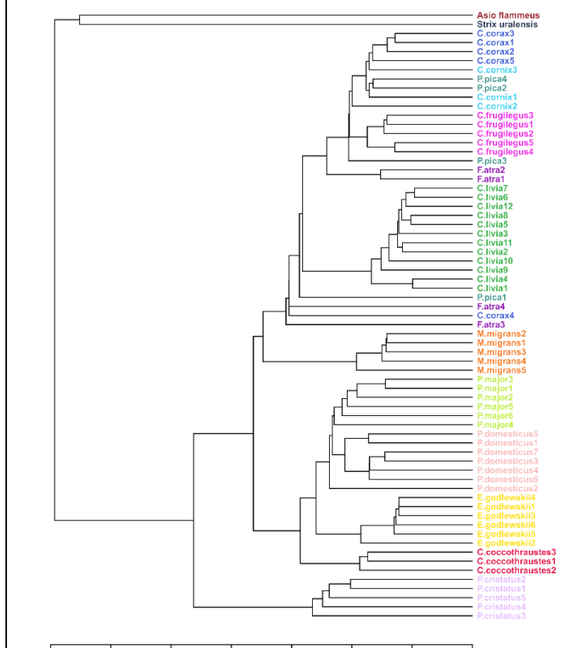
**Pareto-scaled data**

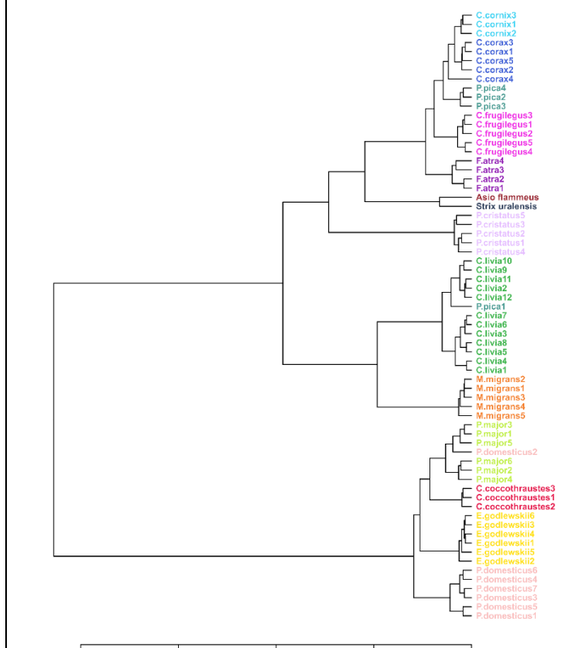
Clustering algorithm

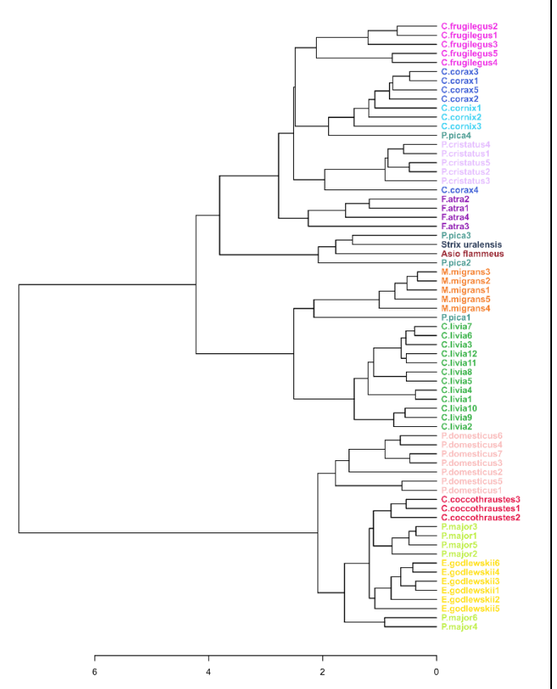
| Similarity measure | Average linkage | Complete linkage | Single linkage | Ward's linkage |
|---|---|---|---|---|
| Euclidean distance | PEA | PEC | PES | PEW |
| Pearson's correlation | PPA | PPC | PPS | PPW |
| Spearman's rank correlation | PSA | PSC | PSS | PSW |

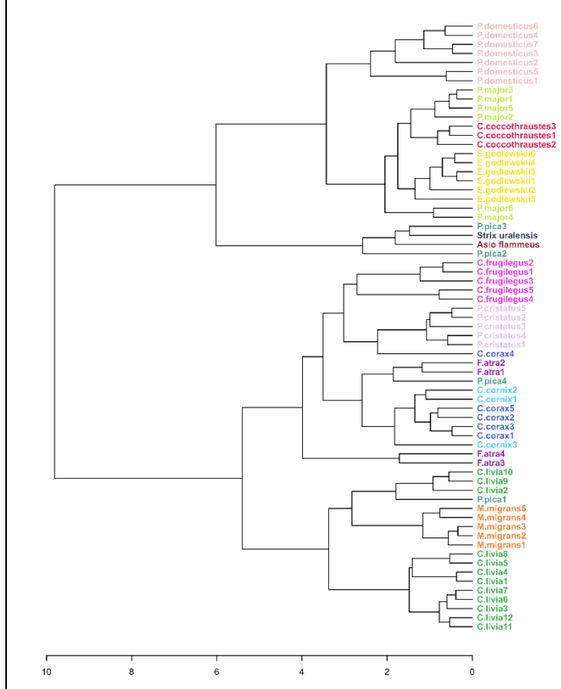


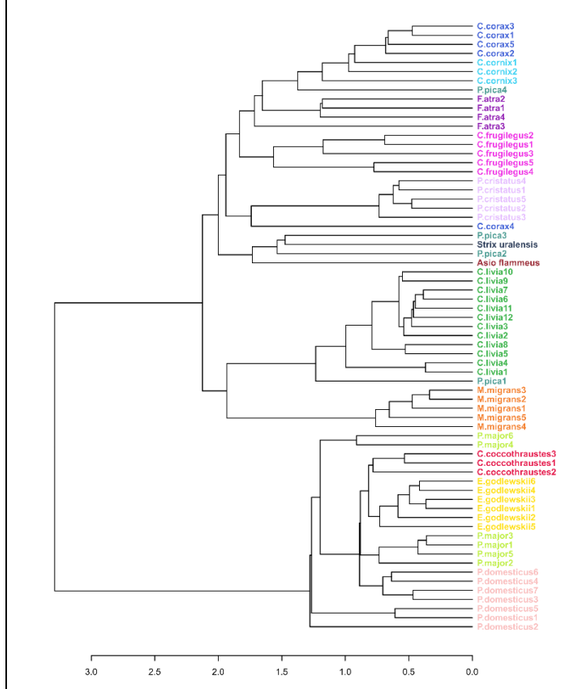



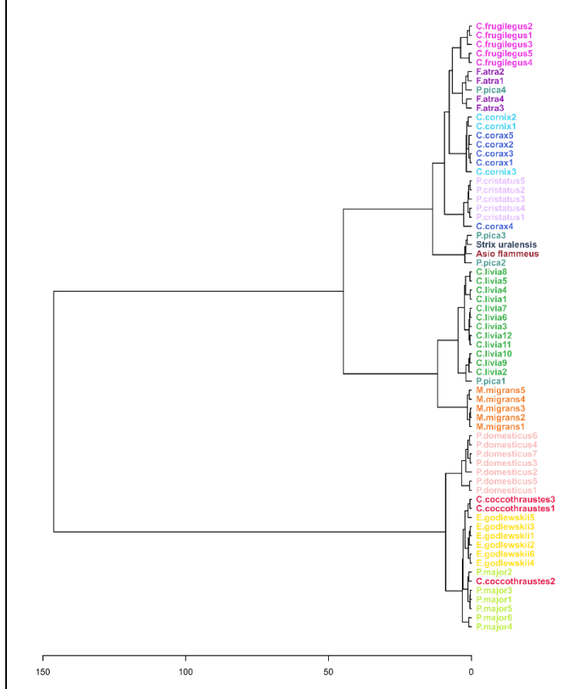



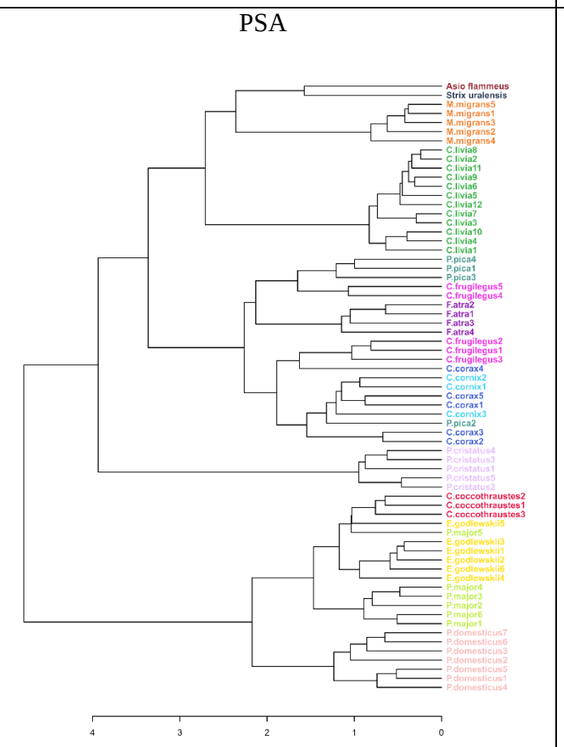



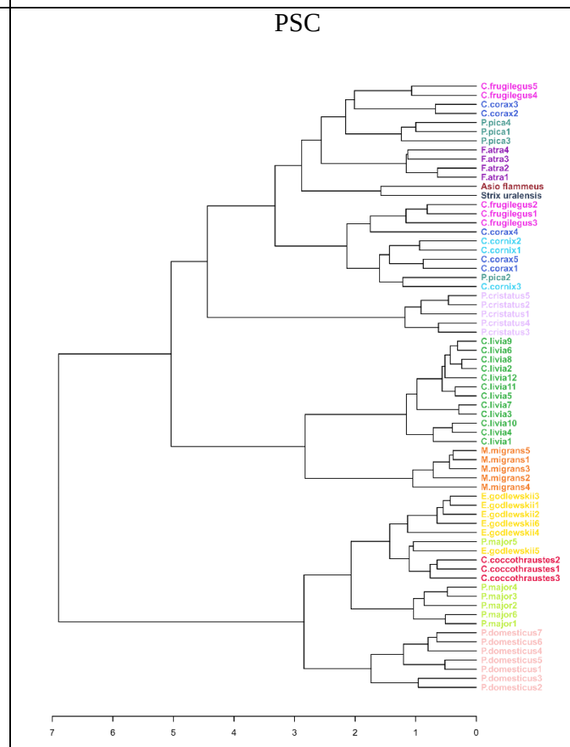



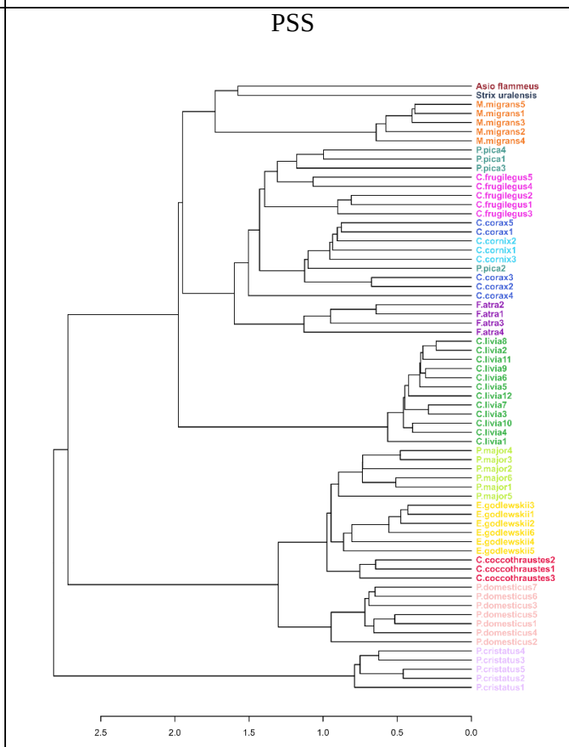



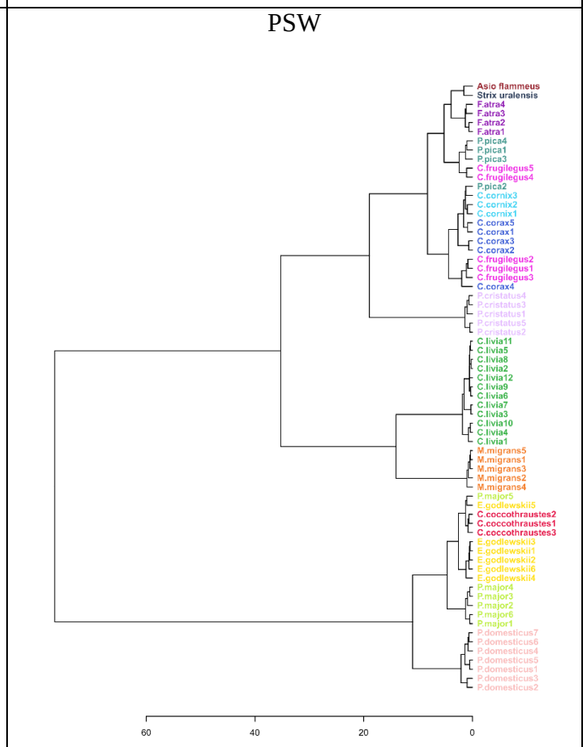



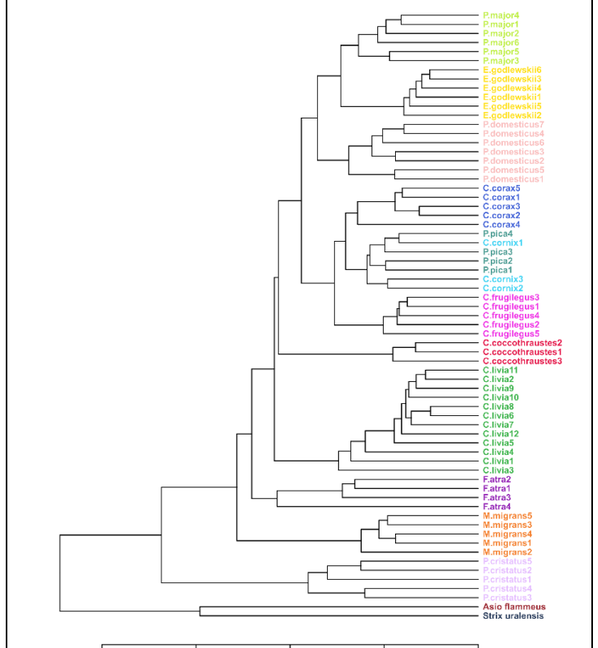
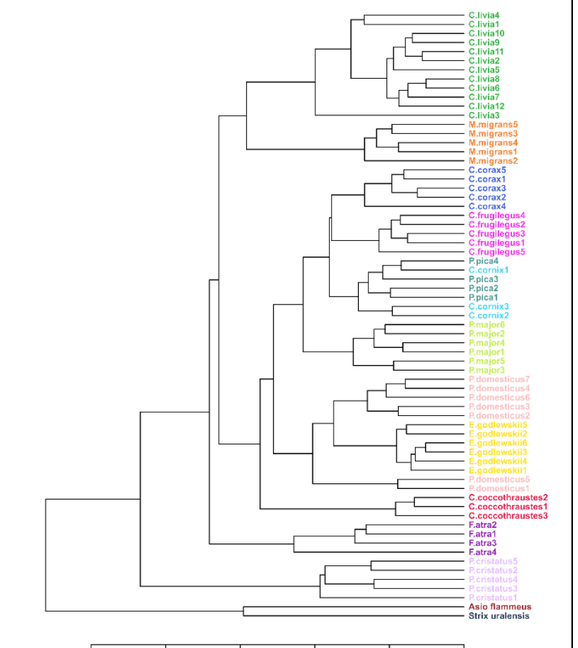
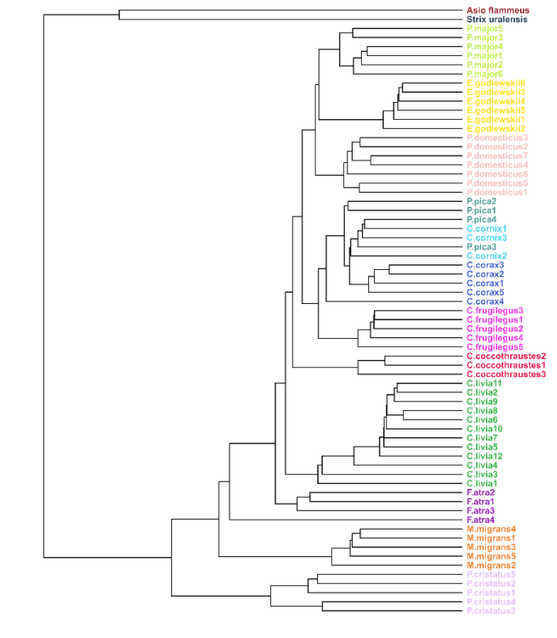
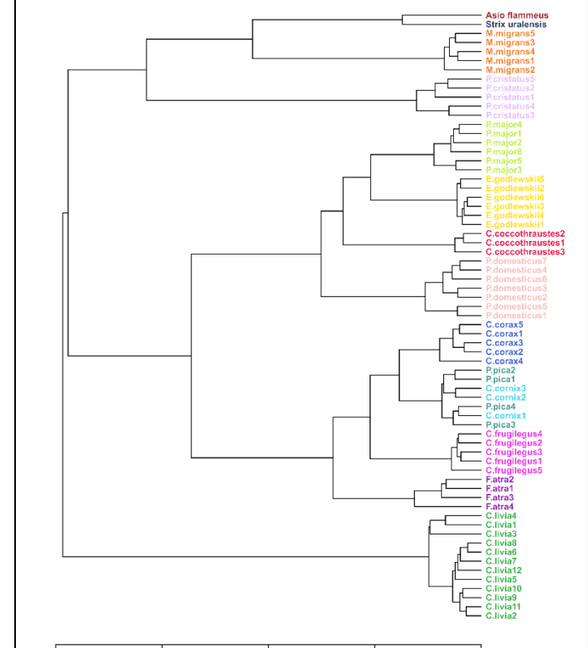
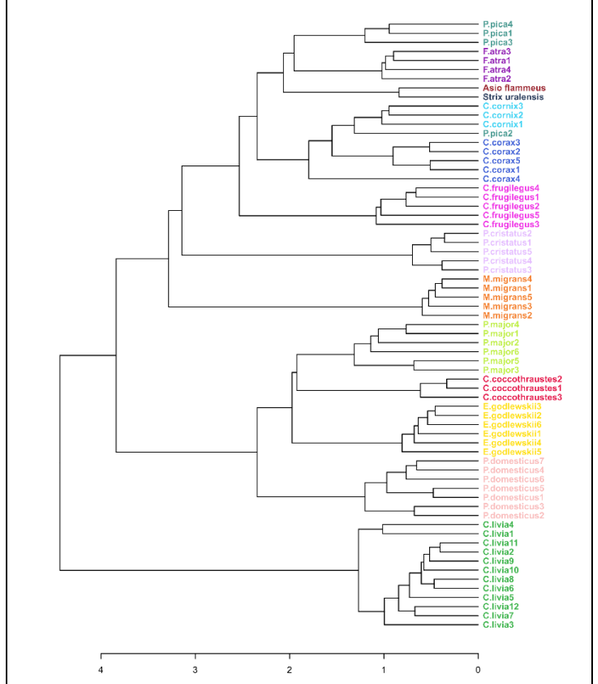
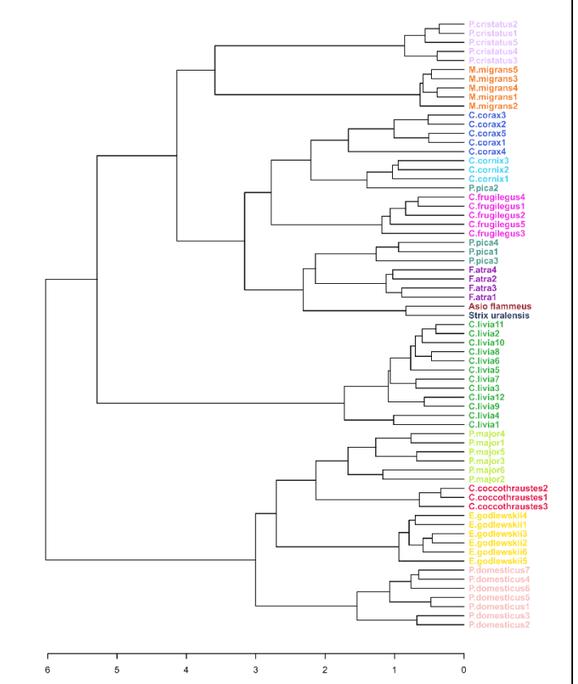
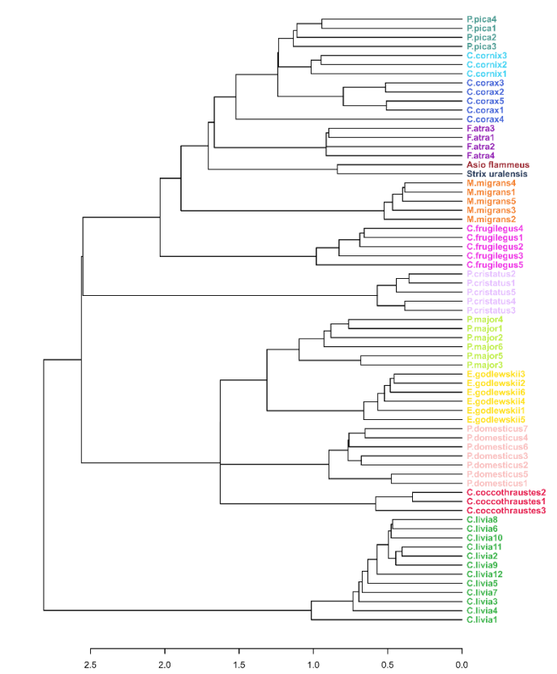
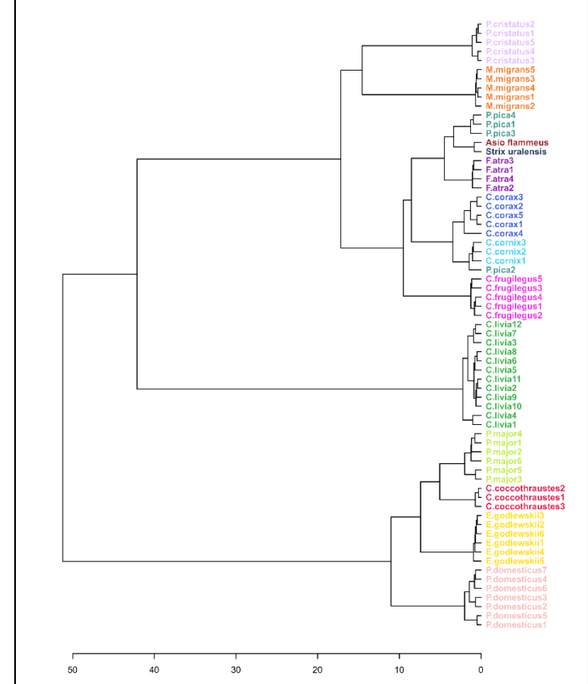
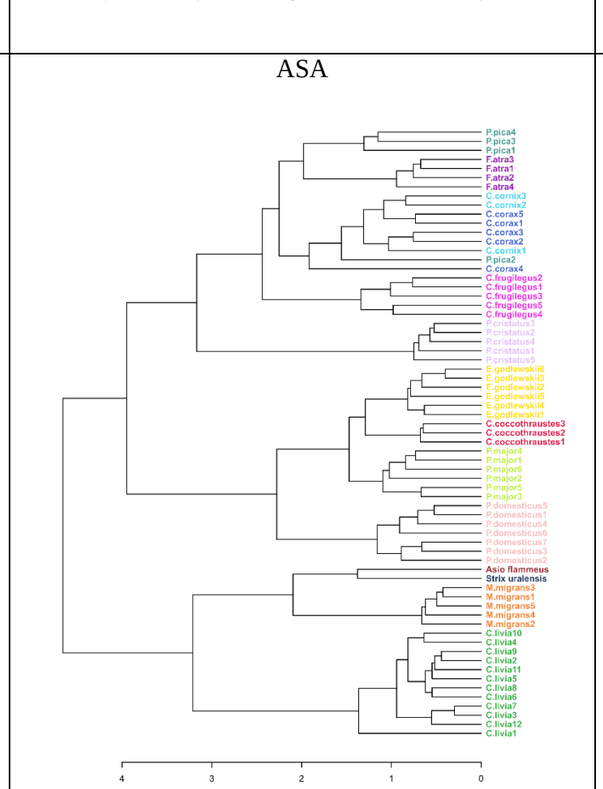
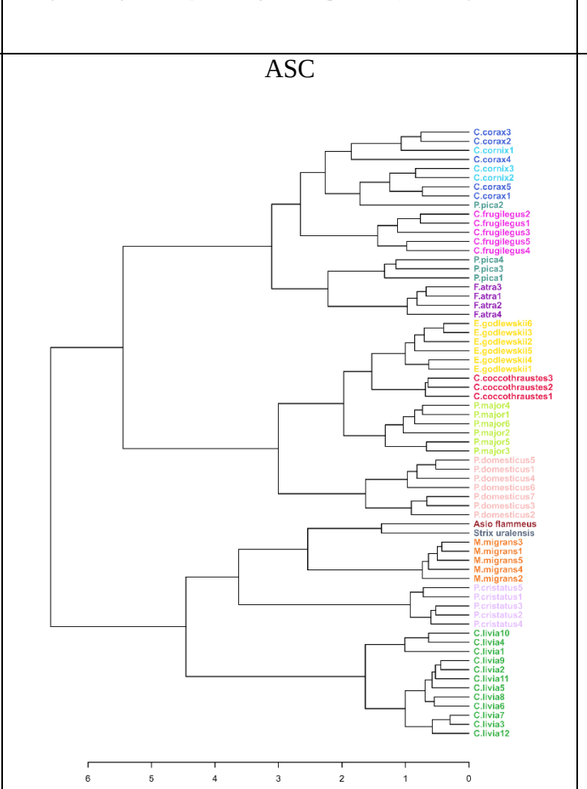
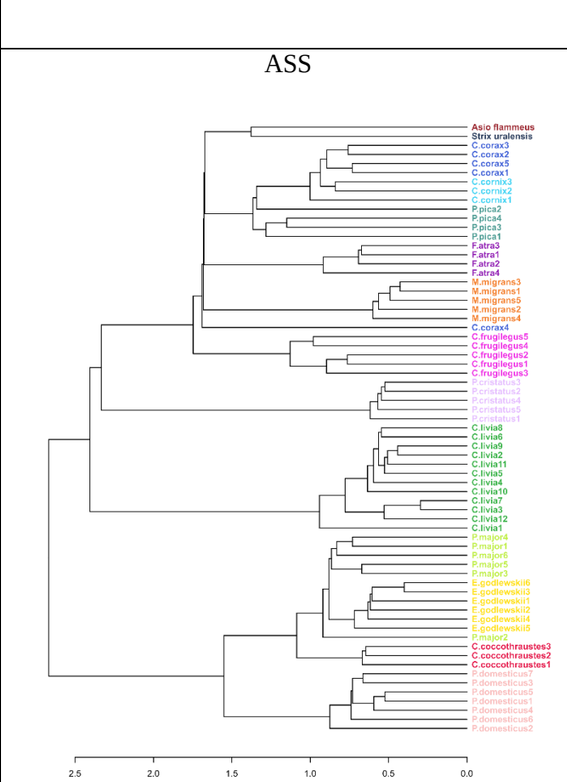
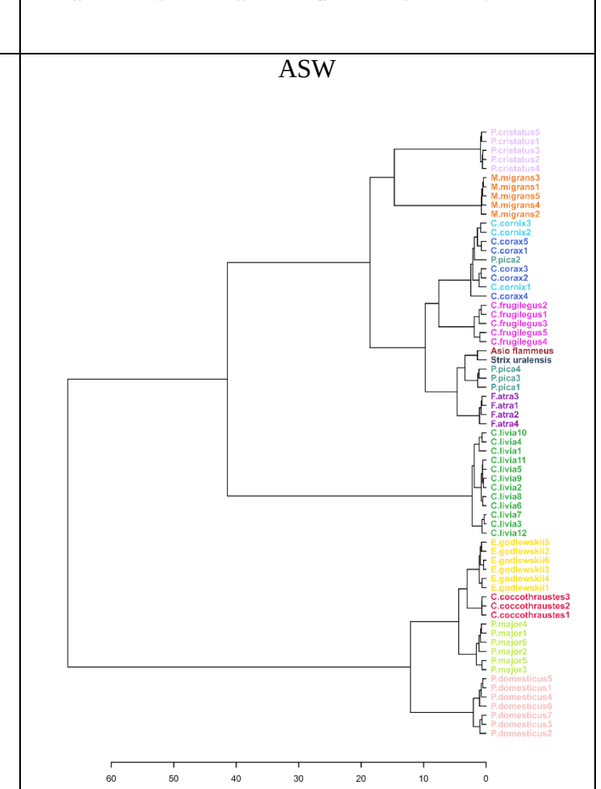



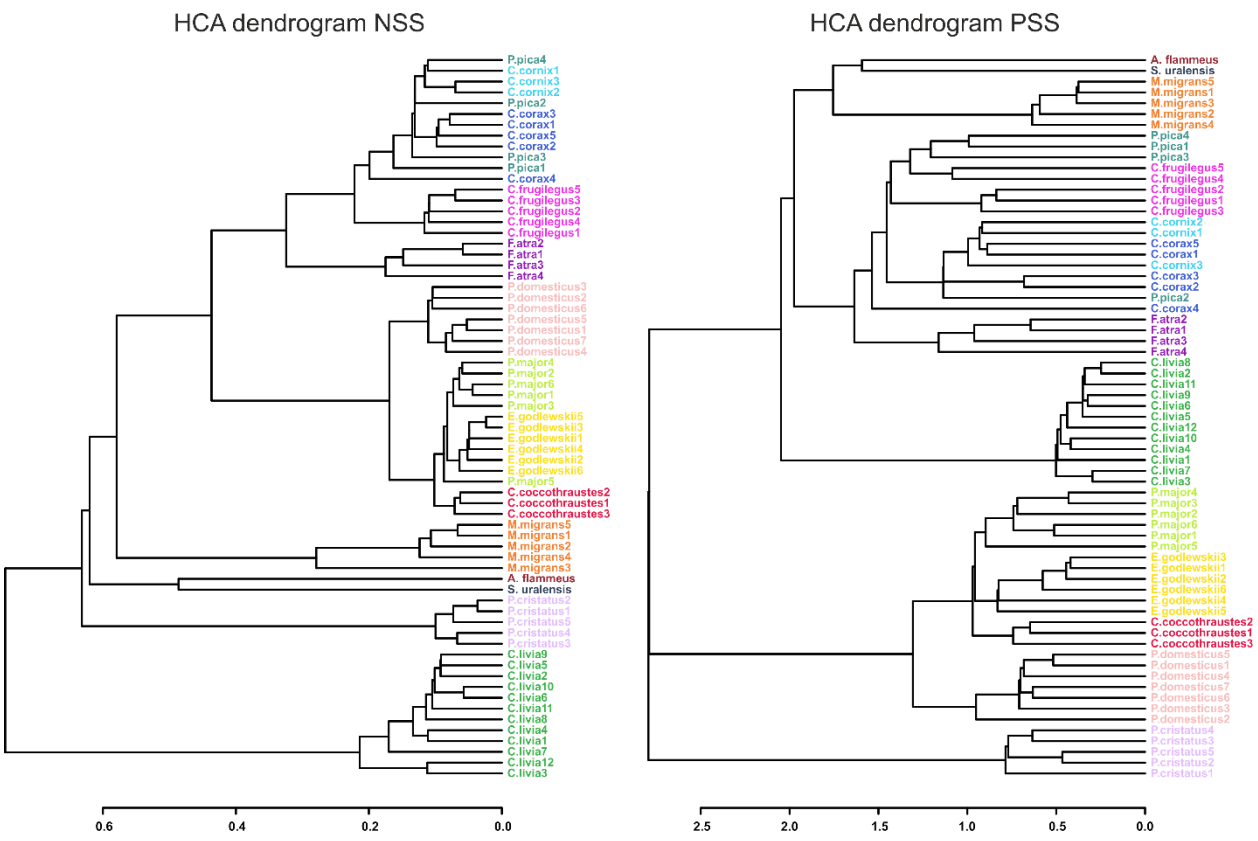



**Auto-scaled data**

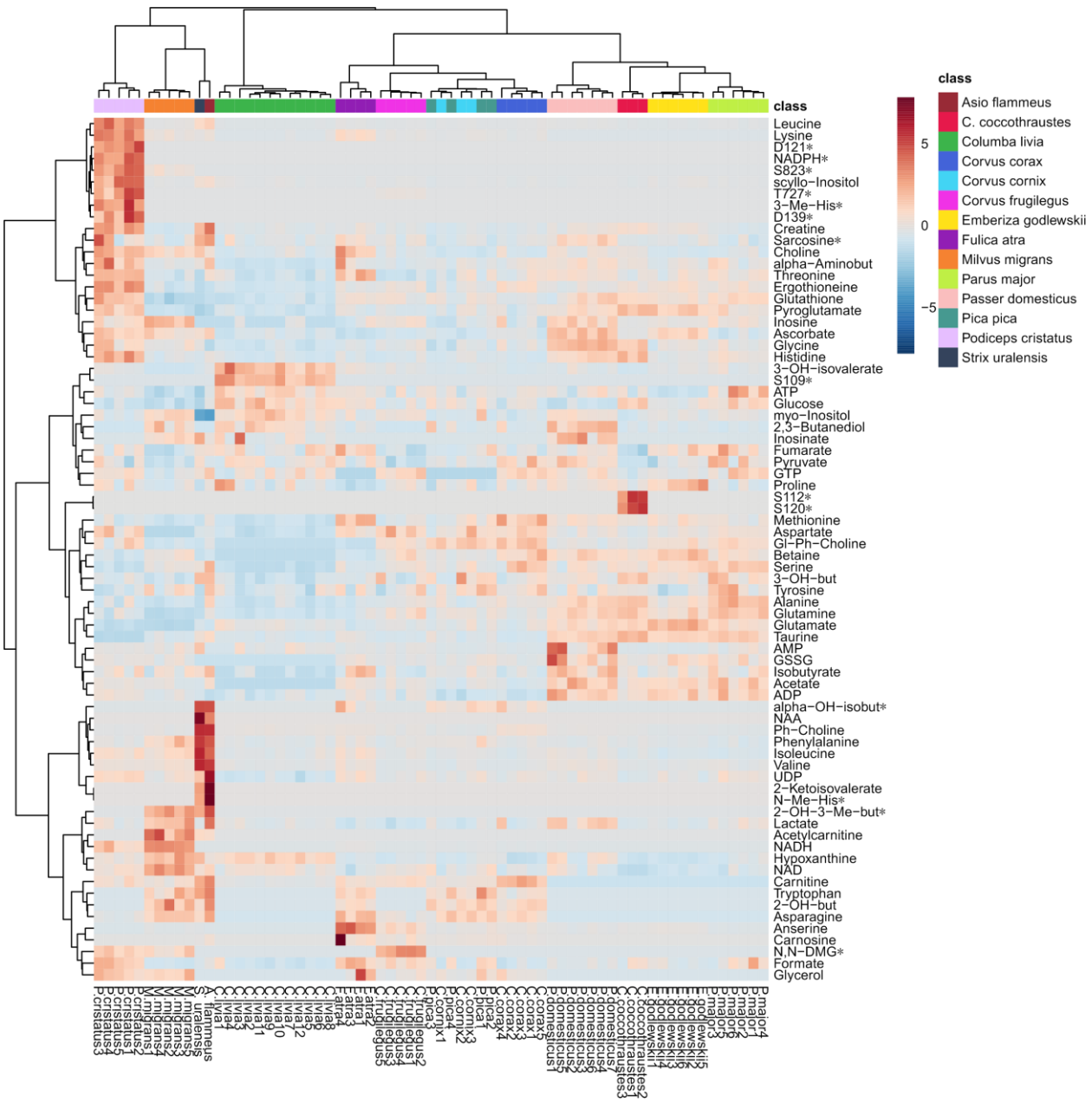
| Similarity measure | Clustering algorithm: Average linkage | Complete linkage | Single linkage | Ward's linkage |
|---|---|---|---|---|
| Euclidean distance | AEA | AEC | AES | AEW |
| Pearson's correlation | APA | APC | APS | APW |
| Spearman's rank correlation | ASA | ASC | ASS | ASW |

**Supplementary Figure S3. HCA clustering results** for all 36 possible combinations of data scalings, similarity measures and clustering algorithms, available in MetaboAnalyst web-platform.




**Supplementary Figure S4. HCA dendrograms obtained for non-scaled (left panel, NSS) or Pareto-scaled (right panel, PSS) data.** Plotted with the Spearman's rank correlation similarity measure and use of Single linkage clustering algorithm (clustering uses the closest pair of observations).




**Supplementary Figure S5. Clustering result shown as heatmap.** HCA is performed for both – metabolites and samples (Euclidean distance measure, and clustering algorithm using Ward's linkage)

## Supplementary Table S2. Sorted average concentrations of metabolites in 14 bird species in nmol/g, color-coded.

**Legend for concentration values coloring:** 20000 [C] > 10.000 nmol/g; 5000 10.000 > [C] > 3.000 nmol/g; 1500 3.000 > [C] > 1.000 nmol/g; 500 <1000 nmol/g

| \ Species / Metabolite | Black kite | Eurasian magpie | Northern raven | Eurasian coot | Godlewski's bunting | Great crested grebe | Great tit | Hawfinch | Hooded crow | House sparrow | Rock dove | Rook | Short-eared owl[a] | Ural owl[a] | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *M. migrans* | *P. pica* | *C. corax* | *F. atra* | *E. godlewskii* | *P. cristatus* | *P. major* | *C. coccothraustes* | *C. cornix* | *P. domesticus* | *C. livia* | *C. frugilegus* | *A. flammeus* | *S. uralensis* | |
| *myo* -Inositol | 37644 | 29142 | 25982 | 28923 | 28984 | 27729 | 29454 | 25127 | 26315 | 33735 | 37071 | 32297 | 2974 | 5354 | 26481 |
| Taurine | 14986 | 20099 | 12722 | 20142 | 34987 | 3225 | 37936 | 47662 | 15029 | 36307 | 14527 | 13989 | 32320 | 25625 | 23540 |
| Lactate | 13884 | 7065 | 7580 | 5467 | 6415 | 7719 | 8070 | 6130 | 5297 | 11539 | 7060 | 4882 | 20575 | 6728 | 8458 |
| Glutamine | 1741 | 5755 | 5385 | 4431 | 7814 | 4609 | 10687 | 10618 | 6362 | 9249 | 3229 | 7374 | 6111 | 2403 | 6126 |
| Acetate | 4261 | 4252 | 3048 | 6004 | 8072 | 5069 | 9677 | 6236 | 3089 | 10392 | 89 | 4887 | 7166 | 3117 | 5383 |
| Glutathione | 1187 | 3529 | 2940 | 3880 | 3972 | 5993 | 4703 | 4643 | 3574 | 4379 | 2143 | 3638 | 1841 | 1397 | 3416 |
| Alanine | 1709 | 3027 | 3219 | 2106 | 3641 | 2371 | 6012 | 5664 | 2637 | 4672 | 1705 | 2916 | 4919 | 1160 | 3268 |
| Ergothioneine | 1863 | 1887 | 4486 | 2982 | 4122 | 9107 | 3760 | 3721 | 3239 | 2904 | 1579 | 3561 | 1021 | 361 | 3185 |
| Serine | 2669 | 2214 | 4352 | 2187 | 3853 | 1192 | 4573 | 3125 | 3209 | 2950 | 904 | 2846 | 5582 | 2915 | 3041 |
| ATP[b] | 2241 | 3307 | 3154 | 3327 | 2381 | 3071 | 4459 | 3072 | 2737 | 3241 | 3848 | 3078 | 2900 | 1563 | 3027 |
| Glutamate | 788 | 1805 | 1858 | 1580 | 3866 | 1022 | 2677 | 2985 | 2126 | 2777 | 2020 | 1890 | 1326 | 1625 | 2025 |
| Creatine | 579 | 629 | 751 | 1010 | 1095 | 2752 | 727 | 1416 | 778 | 1077 | 811 | 828 | 3560 | 2294 | 1308 |
| Pyroglutamate | 564 | 801 | 916 | 917 | 1682 | 1706 | 1222 | 1896 | 1100 | 1328 | 651 | 842 | 343 | 174 | 1010 |
| Glucose | 270 | 1402 | 819 | 1389 | 966 | 499 | 1570 | 1916 | 949 | 445 | 1783 | 796 | 958 | 0 | 983 |
| Ph-Choline | 170 | 89 | 548 | 339 | 69 | 114 | 64 | 167 | 96 | 215 | 246 | 203 | 3918 | 3946 | 727 |
| ADP | 558 | 428 | 381 | 627 | 964 | 718 | 998 | 745 | 298 | 1090 | 466 | 563 | 287 | 470 | 614 |
| Glycine | 584 | 312 | 533 | 437 | 533 | 896 | 549 | 950 | 472 | 1119 | 350 | 543 | 153 | 555 | 570 |
| Proline | 477 | 324 | 596 | 263 | 1045 | 683 | 475 | 575 | 692 | 669 | 647 | 563 | 616 | 278 | 565 |
| Betaine | 591 | 378 | 896 | 205 | 1027 | 226 | 642 | 720 | 465 | 804 | 0 | 647 | 232 | 106 | 496 |
| Methionine | 225 | 429 | 963 | 1005 | 593 | 347 | 467 | 498 | 547 | 607 | 196 | 377 | 481 | 167 | 493 |
| Threonine | 300 | 304 | 425 | 818 | 439 | 860 | 505 | 374 | 478 | 539 | 261 | 224 | 540 | 123 | 442 |
| UDP | 330 | 311 | 296 | 414 | 264 | 487 | 343 | 205 | 266 | 306 | 147 | 275 | 1551 | 592 | 413 |
| Leucine | 191 | 105 | 142 | 164 | 98 | 2175 | 134 | 104 | 164 | 165 | 85 | 116 | 954 | 741 | 381 |
| Anserine | 122 | 220 | 285 | 2449 | 262 | 0 | 73 | 203 | 242 | 150 | 0 | 746 | 28 | 131 | 351 |
| GSSG | 332 | 398 | 284 | 377 | 556 | 352 | 505 | 349 | 256 | 940 | 0 | 280 | 0 | 0 | 331 |
| Ascorbate | 391 | 213 | 234 | 123 | 444 | 489 | 188 | 261 | 161 | 546 | 200 | 182 | 482 | 370 | 306 |
| Valine | 213 | 68 | 92 | 197 | 103 | 186 | 115 | 95 | 112 | 171 | 107 | 94 | 1135 | 1231 | 280 |
| 3-OH-but | 96 | 304 | 210 | 169 | 253 | 166 | 365 | 444 | 343 | 237 | 91 | 214 | 484 | 484 | 276 |
| NAD | 502 | 255 | 148 | 122 | 102 | 248 | 134 | 50 | 134 | 185 | 224 | 213 | 319 | 248 | 206 |
| Histidine | 124 | 97 | 90 | 111 | 144 | 498 | 212 | 431 | 137 | 308 | 85 | 105 | 189 | 98 | 188 |
| Gl-Ph-Choline | 53 | 302 | 389 | 20 | 234 | 242 | 273 | 140 | 396 | 138 | 0 | 239 | 141 | 41 | 186 |
| Tyrosine | 196 | 204 | 200 | 178 | 132 | 125 | 229 | 175 | 208 | 167 | 142 | 167 | 263 | 97 | 177 |
| Formate | 75 | 236 | 83 | 325 | 125 | 322 | 218 | 177 | 68 | 129 | 84 | 233 | 153 | 100 | 166 |
| GTP | 118 | 0 | 269 | 0 | 145 | 221 | 259 | 112 | 0 | 211 | 190 | 267 | 316 | 202 | 165 |
| NADH | 1236 | 48 | 17 | 86 | 8 | 563 | 13 | 8 | 28 | 16 | 3 | 0 | 0 | 9 | 145 |
| Aspartate | 0 | 141 | 210 | 178 | 128 | 215 | 156 | 93 | 219 | 127 | 44 | 243 | 100 | 89 | 139 |
| Lysine | 135 | 67 | 62 | 299 | 0 | 912 | 0 | 0 | 65 | 0 | 12 | 77 | 161 | 88 | 134 |
| Phenylalanine | 184 | 101 | 50 | 74 | 39 | 53 | 24 | 49 | 67 | 44 | 41 | 48 | 509 | 578 | 133 |
| *scyllo* -Inositol | 50 | 34 | 45 | 51 | 70 | 749 | 51 | 39 | 44 | 49 | 55 | 54 | 206 | 171 | 119 |
| Hypoxanthine | 252 | 94 | 50 | 81 | 51 | 125 | 84 | 53 | 66 | 137 | 185 | 73 | 125 | 243 | 116 |
| *alpha* -Aminobut | 63 | 56 | 90 | 131 | 72 | 224 | 97 | 91 | 84 | 118 | 20 | 53 | 192 | 90 | 99 |
| Asparagine | 170 | 135 | 118 | 195 | 0 | 37 | 0 | 0 | 148 | 0 | 0 | 54 | 258 | 113 | 88 |
| Isoleucine | 82 | 34 | 34 | 73 | 24 | 78 | 22 | 29 | 42 | 46 | 36 | 40 | 242 | 315 | 78 |
| D139[c] | 0 | 0 | 0 | 0 | 0 | 1085 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 |
| Glycerol | 219 | 120 | 99 | 258 | 0 | 240 | 0 | 0 | 71 | 0 | 0 | 70 | 0 | 0 | 77 |
| Carnosine | 0 | 60 | 41 | 547 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 98 | 117 | 27 | 68 |
| D121[c] | 0 | 0 | 0 | 0 | 0 | 947 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 68 |
| AMP | 60 | 28 | 11 | 31 | 12 | 51 | 60 | 10 | 10 | 284 | 48 | 39 | 0 | 228 | 62 |
| NAA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 274 | 501 | 55 |
| S823[c] | 102 | 0 | 0 | 0 | 0 | 666 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 |
| Carnitine | 43 | 39 | 100 | 51 | 0 | 52 | 0 | 0 | 40 | 0 | 27 | 57 | 144 | 112 | 48 |
| 3-OH-isovalerate | 0 | 34 | 22 | 66 | 48 | 51 | 51 | 10 | 24 | 44 | 192 | 47 | 45 | 21 | 47 |
| Tryptophan | 71 | 95 | 31 | 65 | 0 | 0 | 0 | 0 | 37 | 0 | 0 | 0 | 161 | 128 | 42 |
| Choline | 54 | 11 | 19 | 78 | 13 | 85 | 20 | 32 | 9 | 34 | 18 | 22 | 41 | 69 | 36 |
| 2,3-Butanediol | 91 | 56 | 31 | 21 | 19 | 0 | 11 | 11 | 10 | 132 | 78 | 0 | 0 | 0 | 33 |
| Inosine | 89 | 17 | 27 | 24 | 34 | 52 | 42 | 29 | 18 | 66 | 8 | 44 | 0 | 0 | 32 |
| T727[c] | 0 | 0 | 0 | 0 | 0 | 399 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 31 |
| Fumarate | 13 | 23 | 19 | 36 | 29 | 24 | 32 | 10 | 18 | 24 | 25 | 14 | 27 | 26 | 23 |
| NADPH[c] | 0 | 0 | 0 | 0 | 0 | 302 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| N-Me-His[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 198 | 80 | 20 |
| Sarcosine[c] | 7 | 4 | 8 | 15 | 8 | 22 | 6 | 7 | 8 | 18 | 7 | 10 | 35 | 30 | 13 |
| *alpha* -OH-isobut[c] | 0 | 11 | 11 | 14 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 60 | 61 | 12 |
| Pyruvate | 7 | 9 | 16 | 12 | 9 | 11 | 16 | 7 | 11 | 15 | 13 | 7 | 9 | 14 | 11 |
| Inosinate | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 15 | 0 | 11 | 58 | 11 |
| 2-OH-but | 28 | 18 | 16 | 14 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 40 | 13 | 10 |
| N,N-DMG[c] | 30 | 0 | 0 | 0 | 0 | 39 | 0 | 0 | 0 | 0 | 0 | 72 | 0 | 0 | 10 |
| Isobutyrate | 8 | 7 | 5 | 15 | 9 | 10 | 10 | 7 | 8 | 16 | 2 | 4 | 19 | 11 | 9 |
| S120[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 126 | 0 | 0 | 0 | 0 | 0 | 0 | 9 |
| Acetylcarnitine | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 22 | 7 |
| 3-Me-His[c] | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| S109[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 95 | 0 | 0 | 0 | 7 |
| S112[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 81 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 2-OH-3-Me-but[c] | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 18 | 5 |
| 2-Ketoisovalerate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 9 | 2 |

[a] Lens from only one individual was analyzed

[b] **abbreviations:** 2-OH-3-Me-but – 2-hydroxy-3-methyl-butyrate; 2-OH-but – 2-hydroxy-butyrate; 3-Me-His – 3-methylhistidine; 3-OH-but – 3-hydroxy-butyrate; 3-OH-isovalerate – 3-hydroxy-isovalerate; ADP – adenosine diphosphate; alpha-Aminobut – alpha-aminobutyrate; alpha-OH-isobut – alpha-hydroxy-isobutyrate; AMP – adenosine monophosphate; ATP – adenosine triphosphate; Gl-Ph-Choline – glycerophosphocholine; GSSG – glutathione oxidized; GTP – guanosine triphosphate; N,N-DMG – N,N-dimethylglycine; NAA – N-acetyl-aspartate; NAD – nicotinamide adenine dinucleotide; NADH – nicotinamide adenine dinucleotide reduced; NADPH – nicotinamide adenine dinucleotide phosphate reduced; N-Me-His – N-methylhistidine; Ph-Choline – phosphocholine; UDP – uridine diphosphate

[c] Low metabolite identification confidence, not confirmed by chemical standards. Concentrations of unknowns were estimated assuming that the signals in the aliphatic part of NMR spectra (S109, S112, S120, D121, and D139) correspond to a single $CH_3$ group, while the aromatic signals (T727 and S823) correspond to CH group.