

## Plötner et al.\_Supplementary Material 1 (Tables S1–S12)

**Table S1.** Parameters of *P. lessonae* (LL) and *P. ridibundus* (RR) genome assemblies.

| Parameter                                      | Assembly    |             |             |
|------------------------------------------------|-------------|-------------|-------------|
|                                                | LL-1        | LL-2        | RR          |
| Size (including Ns) [bp]                       | 4894985653  | 6333496276  | 7162657451  |
| Size (without Ns) [bp]                         | 4041398048  | 4247649372  | 2811228707  |
| Gap Content [%]                                | 17.44       | 32.93       | 60.75       |
| Singletons [N]                                 | 2844675     | 2778275     | 10787472    |
| Scaffolds [N]                                  | 3453250     | 3155940     | 12064811    |
| Mean Size [bp]                                 | 1417        | 2006        | 593         |
| Longest Sequence [bp]                          | 501104      | 1433414     | 73247       |
| Average Length of Breaks (N) in Scaffolds [bp] | 247         | 660         | 360         |
| N50                                            | 27865       | 136366      | 10022       |
| N90                                            | 1405        | 8363        | 154         |
| Scaffolds >1Kbp [N/%]                          | 273239/7.91 | 120999/3.83 | 593941/4.91 |
| Predicted coding sequence [Mbp]                | 380         |             | 78          |

**Table S2.** Gametogenic genes with full names and GenBank accession numbers of their *Xenopus tropicalis* or *Mus musculus* orthologs (marked with an asterisk).

| Gene            | Full name                                                                | Accession number |
|-----------------|--------------------------------------------------------------------------|------------------|
| <b>adarb1</b>   | Double-stranded RNA-specific editase 1                                   | XM_018097020.1   |
| <b>adcy9</b>    | Adenylate cyclase type 9                                                 | XM_002932430.4   |
| <b>ago2</b>     | Argonaute 2, RISC catalytic component                                    | XM_012964517.2   |
| <b>ago3</b>     | Argonaute 3, RISC catalytic component                                    | XM_002936224.4   |
| <b>ago4</b>     | Argonaute 4, RISC catalytic component                                    | XM_004911574.3   |
| <b>akt1</b>     | RAC-alpha serine/threonine-protein kinase                                | XM_002940717.4   |
| <b>anapc13</b>  | Anaphase promoting complex subunit 13                                    | NP_001017240     |
| <b>ar</b>       | Androgen receptor                                                        | XM_002941842.4   |
| <b>asz1</b>     | Ankyrin repeat, SAM and basic leucine zipper domain-containing protein 1 | XM_012958624.2   |
| <b>atr</b>      | Serine/threonine-protein kinase ATR                                      | XM_012963312.2   |
| <b>aurka</b>    | Aurora kinase A                                                          | NM_001016570.2   |
| <b>bcar1</b>    | Breast cancer anti-estrogen resistance protein 1                         | XM_012960799.2   |
| <b>bcar3</b>    | Breast cancer anti-estrogen resistance protein 3                         | NM_001097265.1   |
| <b>birc5</b>    | Baculoviral IAP repeat-containing protein 5                              | NM_001044454.1   |
| <b>brd2</b>     | Bromodomain testis-specific protein                                      | NM_001134810.1   |
| <b>btrc</b>     | F-box/WD repeat-containing protein 1A                                    | XM_012966589.2   |
| <b>bub1</b>     | Mitotic checkpoint serine/threonine-protein kinase BUB1                  | NM_001079362.1   |
| <b>bub3</b>     | Mitotic checkpoint protein BUB3                                          | BC079934.1       |
| <b>calm</b>     | Calmodulin                                                               | NM_001008159.1   |
| <b>camk2g</b>   | Calcium/calmodulin-dependent protein kinase type II subunit gamma        | XM_012966016.2   |
| <b>ccna1</b>    | Cyclin-A1                                                                | NM_001016239.1   |
| <b>ccnb1</b>    | G2/mitotic-specific cyclin-B1                                            | NM_203790.1      |
| <b>ccnb1ip1</b> | E3 ubiquitin-protein ligase CCNB1IP1                                     | XM_018089685.1   |
| <b>ccnb2</b>    | G2/mitotic-specific cyclin-B2                                            | NM_001145376.2   |
| <b>ccnb3</b>    | G2/mitotic-specific cyclin-B3                                            | XM_002939199.4   |
| <b>ccne2</b>    | G1/S-specific cyclin-E2                                                  | NM_001016267.2   |
| <b>cdc20</b>    | Cell division cycle protein 20 homolog                                   | NM_203614.1      |
| <b>cdc25c</b>   | M-phase inducer phosphatase 3                                            | XM_018091902.1   |
| <b>cdk1</b>     | Cyclin-dependent kinase 1                                                | NM_001145376.2   |
| <b>cdk2</b>     | Cyclin-dependent kinase 2                                                | NM_001008135.1   |
| <b>cdk7</b>     | Cyclin-dependent kinase 7                                                | NM_001017219.2   |
| <b>cpeb4</b>    | Cytoplasmic polyadenylation element-binding protein 4                    | XM_002936699.4   |
| <b>ctcf</b>     | Transcriptional repressor CTCF                                           | NM_001122796.1   |
| <b>cyp26b1</b>  | Cytochrome P450 26B1                                                     | NM_001079187.2   |
| <b>dazl</b>     | Deleted in azoospermia-like                                              | NM_203748.1      |
| <b>ddx19</b>    | ATP-dependent RNA helicase DDX19                                         | CR760667.2       |
| <b>ddx25</b>    | ATP-dependent RNA helicase DDX25                                         | NM_001016345.2   |
| <b>ddx4</b>     | Probable ATP-dependent RNA helicase DDX4                                 | XM_018093500.1   |
| <b>dmc1</b>     | Meiotic recombination protein DMC1/LIM15 homolog                         | XM_018093651.1   |
| <b>dmrt1</b>    | Doublesex and mab-3 related transcription factor 1                       | XM_018089347.1   |
| <b>dmrt2</b>    | Doublesex and mab-3 related transcription factor 2                       | NM_001100256.1   |
| <b>dmrt5</b>    | Doublesex and mab-3 related transcription factor 5                       | HQ171197.1       |
| <b>dnmt1</b>    | DNA (cytosine-5)-methyltransferase 1                                     | XM_002934338.4   |
| <b>dnmt3a</b>   | DNA (cytosine-5)-methyltransferase 3A                                    | XM_004919615.3   |
| <b>dusp1</b>    | Dual specificity protein phosphatase 1                                   | NM_001005450.2   |
| <b>eif4g3</b>   | Eukaryotic translation initiation factor 4 gamma 3                       | NM_001142072.1   |
| <b>eme1</b>     | Crossover junction endonuclease EME1                                     | NM_001078831.1   |
| <b>eme2</b>     | Probable crossover junction endonuclease EME2                            | NM_001079392.1   |
| <b>exd1</b>     | piRNA biogenesis protein EXD1                                            | XM_004917310.3   |
| <b>fbxo43</b>   | F-box only protein 43                                                    | XM_004915140.3   |
| <b>fbxo5</b>    | F-box only protein 5                                                     | XM_012963092.1   |
| <b>fgf9</b>     | Fibroblast growth factor 9                                               | XM_002938575.4   |
| <b>fkbp6</b>    | Inactive peptidyl-prolyl cis-trans isomerase FKBP6                       | XM_018091140.1   |
| <b>foxc1</b>    | Forkhead box protein C1                                                  | NM_001007863.1   |
| <b>grb2</b>     | Growth factor receptor-bound protein 2                                   | NM_001008129.1   |
| <b>h3.3</b>     | Histone H3.3                                                             | NM_001005101.1   |
| <b>hat1</b>     | Histone acetyltransferase type B catalytic subunit                       | XM_012970071.2   |
| <b>hdac1</b>    | Histone deacetylase 1                                                    | NM_001030393.1   |
| <b>hec1</b>     | Kinetochore protein NDC80 homolog                                        | NM_001011162.1   |
| <b>henmt1</b>   | Small RNA 2'-O-methyltransferase                                         | XM_012960696.2   |
| <b>hormad1</b>  | HORMA domain-containing protein 1                                        | XM_002935467.2   |
| <b>igf1</b>     | Insulin-like growth factor 1                                             | XM_002936829.4   |
| <b>igf1r</b>    | Insulin-like growth factor 1 receptor                                    | XM_002933305.4   |
| <b>ints13</b>   | Integrator complex subunit 13                                            | NM_001004984.1   |
| <b>itpr1</b>    | Inositol 1,4,5-trisphosphate receptor type 1                             | XM_012961730.2   |
| <b>kif22</b>    | Kinesin-like protein KIF22                                               | XM_012954607.2   |
| <b>kmt2a</b>    | Histone-lysine N-methyltransferase 2A                                    | XM_018096068.1   |
| <b>m1ap</b>     | Meiosis 1 arrest protein                                                 | NM_001130377.1   |

| Gene            | Full name                                                                         | Accession number |
|-----------------|-----------------------------------------------------------------------------------|------------------|
| <i>mad211</i>   | Mitotic spindle assembly checkpoint protein MAD2A                                 | NM_001103058.1   |
| <i>mael</i>     | Protein maelstrom homolog                                                         | NM_001079177.1   |
| <i>map2k1</i>   | Dual specificity mitogen-activated protein kinase kinase 1                        | NM_001008057.1   |
| <i>map3k7</i>   | Mitogen-activated protein kinase kinase kinase 7                                  | XM_018093745.1   |
| <i>mapk1</i>    | Mitogen-activated protein kinase 1                                                | NM_001017127.2   |
| <i>mapk12</i>   | Mitogen-activated protein kinase 12                                               | XM_018092105.1   |
| <i>marf1</i>    | Meiosis regulator and mRNA stability factor 1                                     | NM_001126066.1   |
| <i>mdc1</i>     | Mediator of DNA damage checkpoint protein 1                                       | XM_012969222.2   |
| <i>mei4</i>     | Meiosis-specific protein MEI4                                                     | XM_018094012.1   |
| <i>meig1</i>    | Meiosis-expressed gene 1 protein homolog                                          | NM_001016522.2   |
| <i>meiob</i>    | Meiosis-specific with OB domain-containing protein                                | XM_012970697.2   |
| <i>mlh1</i>     | DNA mismatch repair protein Mlh1                                                  | XM_002933409.3   |
| <i>mlh3</i>     | DNA mismatch repair protein Mlh3                                                  | NM_001251842.2   |
| <i>mnd1</i>     | Meiotic nuclear division protein 1 homolog                                        | XM_012961773.1   |
| <i>mns1</i>     | Meiosis-specific nuclear structural protein 1                                     | NM_001103012.2   |
| <i>mov10l1</i>  | RNA helicase Mov10l1                                                              | NM_001079156.1   |
| <i>mre11</i>    | Double-strand break repair protein MRE11                                          | XM_012956402.2   |
| <i>msh2</i>     | DNA mismatch repair protein Msh2                                                  | XM_002935381.4   |
| <i>msh4</i>     | MutS protein homolog 4                                                            | XM_018093532.1   |
| <i>msh5</i>     | MutS protein homolog 5                                                            | XM_018096397.1   |
| <i>msh6</i>     | DNA mismatch repair protein Msh6                                                  | XM_002935375.3   |
| <i>msx1</i>     | Homeobox protein MSX-1                                                            | NM_001037252.1   |
| <i>mus81</i>    | Crossover junction endonuclease MUS81                                             | NM_001011004.1   |
| <i>mybl1</i>    | Myb-related protein A                                                             | XM_002935319.4   |
| <i>nbn</i>      | Nibrin                                                                            | XM_018095158.1   |
| <i>nusap1</i>   | Nucleolar and spindle-associated protein 1                                        | XM_012968505.2   |
| <i>parn</i>     | Poly(A)-specific ribonuclease PARN                                                | XM_012970508.2   |
| <i>pelp1</i>    | Proline-, glutamic acid- and leucine-rich protein 1                               | XM_012953417.2   |
| <i>piwil2</i>   | Piwi-like protein 2                                                               | XM_018091922.1   |
| <i>piwil4</i>   | Piwi-like protein 4                                                               | XM_012958238.1   |
| <i>pkmyt1</i>   | Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase       | XM_012970107.2   |
| <i>pld6</i>     | Mitochondrial cardiolipin hydrolase                                               | NM_001016875.3   |
| <i>plk1</i>     | Serine/threonine-protein kinase PLK1                                              | NM_213679.2      |
| <i>pms1</i>     | PMS1 protein homolog 1                                                            | NM_001142901.2   |
| <i>pms2</i>     | Mismatch repair endonuclease PMS2                                                 | XM_002931979.4   |
| <i>ppp1ca</i>   | Serine/threonine-protein phosphatase PP1-alpha catalytic subunit                  | NM_001127010.1   |
| <i>ppp2r1a</i>  | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform | NM_204074.1      |
| <i>ppp2r5a</i>  | Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha isoform   | NM_001078689.1   |
| <i>ppp3ca</i>   | Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform           | XM_012955571.2   |
| <i>prc1</i>     | Protein regulator of cytokinesis 1                                                | XM_012958665.2   |
| <i>prdm9</i>    | Histone-lysine N-methyltransferase PRDM9                                          | NM_001113936.1   |
| <i>prkaca</i>   | cAMP-dependent protein kinase catalytic subunit alpha                             | XM_018092865.1   |
| <i>psmc3ip</i>  | Homologous-pairing protein 2 homolog                                              | XM_012966161.1   |
| <i>pttg1</i>    | Securin                                                                           | XM_004913488.3   |
| <i>rad21</i>    | Double-strand-break repair protein rad21 homolog                                  | XM_012965196.2   |
| <i>rad50</i>    | DNA repair protein RAD50                                                          | XM_012959771.2   |
| <i>rad51</i>    | DNA repair protein RAD51 homolog 1                                                | NM_001016393.3   |
| <i>rad51ap1</i> | RAD51-associated protein 1                                                        | XM_002940303.4   |
| <i>rad52</i>    | DNA repair protein RAD52 homolog                                                  | NM_001097220.1   |
| <i>rad54l</i>   | DNA repair and recombination protein RAD54-like                                   | NM_001001241.2   |
| <i>rbbp8</i>    | DNA endonuclease RBBP8                                                            | XM_012965325.2   |
| <i>rec114</i>   | Meiotic recombination protein REC114                                              | NM_028598.1 *    |
| <i>rec8</i>     | Meiotic recombination protein REC8 homolog                                        | NM_001079137.1   |
| <i>recq1</i>    | ATP-dependent DNA helicase Q1                                                     | NM_001016316.2   |
| <i>rpa1</i>     | Replication protein A 70 kDa DNA-binding subunit                                  | NM_001015732.1   |
| <i>rpa2</i>     | Replication protein A 32 kDa subunit                                              | NM_001006794.1   |
| <i>rpa3</i>     | Replication protein A 14 kDa subunit                                              | NM_001016626.2   |
| <i>rps6ka3</i>  | Ribosomal protein S6 kinase alpha-3                                               | XM_002936044.4   |
| <i>sfr1</i>     | Swi5-dependent recombination DNA repair protein 1 homolog                         | XM_012967754.2   |
| <i>sgo1</i>     | Shugoshin 1                                                                       | XM_012964257.2   |
| <i>skp1</i>     | S-phase kinase-associated protein 1                                               | XM_012959026.2   |
| <i>smc1a</i>    | Structural maintenance of chromosomes protein 1A                                  | XM_002935514.3   |
| <i>smc1b</i>    | Structural maintenance of chromosomes protein 1B                                  | NM_001278349.2   |
| <i>smc3</i>     | Structural maintenance of chromosomes protein 3                                   | XM_002939498.4   |
| <i>spata1</i>   | Spermatogenesis-associated protein 1                                              | XM_002931696.4   |
| <i>spata2</i>   | Spermatogenesis-associated protein 2                                              | XM_002933154.4   |
| <i>spata22</i>  | Spermatogenesis-associated protein 22                                             | XM_012957517.2   |
| <i>spata4</i>   | Spermatogenesis-associated protein 4                                              | XM_002933324.4   |
| <i>spata5</i>   | Spermatogenesis-associated protein 5                                              | XM_004911116.3   |
| <i>spdyd</i>    | Speedy protein C                                                                  | XM_004913654.3   |
| <i>spo11</i>    | Meiotic recombination protein SPO11                                               | NM_001008199.1   |

| Gene           | Full name                                          | Accession number |
|----------------|----------------------------------------------------|------------------|
| <i>stra8</i>   | Stimulated by retinoic acid gene 8 protein homolog | XM_018089880.1   |
| <i>sumo1</i>   | Small ubiquitin-related modifier 1                 | NM_001005111.1   |
| <i>sun1</i>    | SUN domain-containing protein 1                    | XM_012971093.2   |
| <i>suv39h1</i> | Histone-lysine N-methyltransferase SUV39H1         | XM_002944399     |
| <i>syce2</i>   | Synaptonemal complex central element protein 2     | XM_018092692.1   |
| <i>syce3</i>   | Synaptonemal complex central element protein 3     | XM_002939528.2   |
| <i>sycp1</i>   | Synaptonemal complex protein 1                     | XM_018091546.1   |
| <i>sycp3</i>   | Synaptonemal complex protein 3                     | NM_001078758.1   |
| <i>tdrd1</i>   | Tudor domain-containing protein 1                  | XM_004919374.3   |
| <i>tdrd3</i>   | Tudor domain-containing protein 3                  | NM_204054.1      |
| <i>tdrd5</i>   | Tudor domain-containing protein 5                  | NM_001079295.1   |
| <i>tdrd9</i>   | Tudor domain-containing protein 9                  | NM_001251817.1   |
| <i>tex11</i>   | Testis-expressed protein 11                        | XM_018096967.1   |
| <i>tex12</i>   | Testis-expressed protein 12                        | AF285582.1 *     |
| <i>tex14</i>   | Testis-expressed protein 14 like                   | NM_001128646.1   |
| <i>topbp1</i>  | DNA topoisomerase 2-binding protein 1              | XM_002937745.3   |
| <i>trip13</i>  | Pachytene checkpoint protein 2 homolog             | XM_012964448.2   |
| <i>ttk</i>     | Dual specificity protein kinase TTK                | XM_012963159.2   |
| <i>wee1</i>    | Wee1-like protein kinase                           | NM_001126965.1   |
| <i>ywhaz</i>   | 14-3-3 protein zeta/delta                          | NM_203842.1      |
| <i>zfp36</i>   | mRNA decay activator protein ZFP36                 | NM_001113071.1   |

**Table S3.** Sampling localities, and genotypes (males, females, \*juveniles or subadults) of the individuals studied. The localities of each particular population system are sorted from north to south.

| Locality                 | Abbr. | Coordinates  |              | Population System | Genotype |        |           |    | Plate |
|--------------------------|-------|--------------|--------------|-------------------|----------|--------|-----------|----|-------|
|                          |       | North        | East         |                   | LL       | RR     | ESC       | ?  |       |
| Cítov [CZ]               | Cit   | 50°21'25.1"N | 14°24'08.2"E | R                 |          | 29,24  |           |    | 1     |
| Želechovice [CZ]         | Zel   | 49°44'57.4"N | 17°08'06.2"E | R                 |          | 10,10  |           |    | 1     |
| Šúr (Svätý Jur) [SK]     | Sur   | 48°13'44.1"N | 17°12'15.4"E | R                 |          | 7,0,1* |           |    | 5     |
| Kapuvár [HU]             | Kap   | 47°40'03.6"N | 17°08'01.7"E | R                 |          | 11,2   |           |    | 2     |
| Oltenița, Danube [RO]    | Olt   | 44°04'38.7"N | 26°37'24.1"E | R                 |          | 4,1,4* |           |    | 3     |
| Veleka, Brodilovo [BU]   | Bul   | 42°05'22.6"N | 27°51'19.2"E | R                 |          | 4,1    |           |    | 3     |
| Elesnitsa [BU]           |       | 41°51'41.0"N | 23°37'49.6"E | R                 |          | 3,0    |           |    | 3     |
| Karsibor [PL]            | Kar   | 53°51'07.7"N | 14°20'05.6"E | R-E               |          | 0,9,1* | 8,1,1*    |    | 2     |
| Friedrichshagen [DE]     |       | 52°26'20.7"N | 13°37'59.9"E | R-E (?)           |          | 6,3    |           |    | 3     |
| Lebus [DE]               | Leb   | 52°25'02.3"N | 14°32'31.1"E | R-E               |          | 49,2   | 13,0      |    | 2     |
| Dolní Benešov [CZ]       | Dol   | 49°55'05.0"N | 18°05'50.7"E | R-E               |          | 1,13   | 13,0      |    | 1,2   |
| Košatka [CZ]             | Kos   | 49°44'03.6"N | 18°09'28.0"E | R-E               |          | 8,16   | 17,0      |    | 1,4   |
| Albrechtický [CZ]        | Alb   | 49°42'21.7"N | 18°05'36.8"E | R-E               |          | 12,3   | 1,0       |    | 4     |
| Borovec [CZ]             | Bor   | 49°37'58.6"N | 18°06'09.0"E | R-E (?)           |          | 6,0    | 7,8       | 1* | 2     |
| Melzower Forst [DE]      |       | 53°10'52.2"N | 13°56'15.2"E | L-E               | 11,9,1*  |        | 13,12,39* |    | 5,6   |
| Untermühle [DE]          | DE    | 53°04'51.9"N | 12°54'00.3"E | L-E               | 1,0      |        | 1,0       |    | 5     |
| Teschendorf [DE]         |       | 52°50'37.2"N | 13°08'52.5"E | L-E               | 6,2      |        | 10,1      |    | 3     |
| Český Těšín [CZ]         | Cet   | 49°44'39.3"N | 18°36'29.0"E | L-E               | 7,2      |        | 0,5       |    | 2,3   |
| Trnávka [CZ]             | Trn   | 49°40'55.7"N | 18°11'00.9"E | L-E               | 6,5      |        | 0,4       |    | 3     |
| Bara-Grubišno [HR]       |       | 45°42'30.9"N | 17°09'02.3"E | L-E               | 0,2      |        | 3,3       |    | 2     |
| Pedersker, Bornholm [DK] |       | 55°02'07.2"N | 14°59'45.0"E | E                 |          |        | 34,11     |    | 6     |
| Fehmarn [DE]             |       | 54°31'10.1"N | 11°03'14.1"E | E                 |          |        | 11,10,2*  |    | 7     |
| Rügen [DE]               |       | 54°25'01.6"N | 13°23'45.9"E | E                 | 1*       |        | 45,31,4*  |    | 6,7   |
| Usedom [DE]              |       | 53°57'12.7"N | 14°05'29.1"E | E                 |          |        | 12,1      |    | 6     |
| Jarzewowo, Wolin [PL]    |       | 53°54'32.6"N | 14°38'45.9"E | E                 |          |        | 4,0,1*    |    | 2     |
| Wysoka Kamińska [PL]     |       | 53°49'53.2"N | 14°51'51.8"E | E                 |          |        | 10,11,1*  |    | 2,6   |

Population system: R: all-*P. ridibundus* population, R-E: *P. ridibundus*-*P. esculentus* population, L-E: *P. lessonae*-*P. esculentus* population, E: all-hybrid (*P. esculentus*) population, ?: classification uncertain.

Genotype: LL: *P. lessonae*, RR: *P. ridibundus*, ESC: *P. esculentus*, \*juveniles and subadults of unspecified sex.

Plate: corresponding numbers of the well-plate used for GT-seq sequencing (see Supplementary Material 8)

Country abbreviations: BU: Bulgaria, CZ: Czech Republic, DE: Germany, DK: Denmark, HR: Croatia, HU: Hungary, PL: Poland, RO: Romania, SK: Slovakia.

**Table S4.** Single nucleotide polymorphisms (SNP) and corresponding amino acid substitutions (aa) detected by GT-Seq in 52 gametogenic genes of *P. lessonae* (LL) and *P. ridibundus* (RR). SNP alleles considered to be species-specific are shown in bold, putatively introgressed alleles are marked with asterisks. Nucleotide (nu) T of SNP59 was observed only in *P. esculentus* (genotype LR). Country abbreviations: CZ - Czech Republic, HR - Croatia, DE - Germany.

| SNP | Gene           | Exon | Substitution |            | SNP alleles  |          |              |
|-----|----------------|------|--------------|------------|--------------|----------|--------------|
|     |                |      | nt           | aa         | LL [CZ, HR]  | LL [DE]  | RR           |
| 1   | <i>adcy9</i>   | 6    | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G, A*</b> |
| 2   | <i>adcy9</i>   | 6    | C/T          | -          | <b>C</b>     | <b>C</b> | <b>T, C*</b> |
| 3   | <i>brd2</i>    | 2    | C/T          | -          | T            | T        | C, T         |
| 4   | <i>brd2</i>    | 4    | A/G          | -          | A            | A        | A, G         |
| 5   | <i>brd2</i>    | 5    | A/T          | -          | A            | A        | A, T         |
| 6   | <i>btrc</i>    | 6    | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G</b>     |
| 7   | <i>bub3</i>    | 1    | A/G          | -          | <b>G</b>     | <b>G</b> | <b>A</b>     |
| 8   | <i>bub3</i>    | 3    | C/T          | -          | C            | C        | C, T         |
| 9   | <i>camk2g</i>  | 19   | C/T          | -          | C            | C        | C, T         |
| 10  | <i>camk2g</i>  | 19   | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 11  | <i>camk2g</i>  | 19   | A/C          | -          | <b>A</b>     | <b>A</b> | <b>C</b>     |
| 12  | <i>cdc20</i>   | 1    | C/T          | -          | C            | C        | C, T         |
| 13  | <i>cdc25c1</i> | 10   | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G</b>     |
| 14  | <i>cdk2</i>    | 4    | A/T          | -          | <b>A</b>     | <b>A</b> | <b>T</b>     |
| 15  | <i>cdk2</i>    | 6    | G/T          | -          | <b>G</b>     | <b>G</b> | <b>T</b>     |
| 16  | <i>cpeb4</i>   | 1    | A/G          | -          | A            | A        | A, G         |
| 17  | <i>cpeb4</i>   | 7    | C/T          | -          | C            | C        | C, T         |
| 18  | <i>ctcf</i>    | 1    | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 19  | <i>ctcf</i>    | 1    | A/G          | -          | G            | G        | A, G         |
| 20  | <i>fbxo43</i>  | 2    | A/G          | <b>G/R</b> | <b>G</b>     | <b>G</b> | <b>A</b>     |
| 21  | <i>fbxo43</i>  | 5    | A/C          | -          | <b>A</b>     | <b>A</b> | <b>C</b>     |
| 22  | <i>fbxo43</i>  | 5    | C/T          | -          | C            | C        | C, T         |
| 23  | <i>fbxo5</i>   | 1    | G/T          | -          | G, T         | G, T     | G            |
| 24  | <i>hat1</i>    | 9    | C/T          | -          | <b>C</b>     | <b>C</b> | <b>T, C*</b> |
| 25  | <i>henmt1</i>  | 3    | C/T          | -          | <b>C</b>     | <b>C</b> | <b>T, C*</b> |
| 26  | <i>henmt1</i>  | 4    | C/T          | -          | T            | T        | C, T         |
| 27  | <i>henmt1</i>  | 4    | A/C          | -          | C            | C        | A, C         |
| 28  | <i>henmt1</i>  | 6    | A/G          | A/T        | G            | G        | A, G         |
| 29  | <i>hop2</i>    | 1    | A/C          | -          | <b>C</b>     | <b>C</b> | <b>A</b>     |
| 30  | <i>hormad1</i> | 7    | C/T          | -          | <b>C</b>     | <b>C</b> | <b>T, C*</b> |
| 31  | <i>hormad1</i> | 7    | C/T          | -          | C, T         | C, T     | C, T         |
| 32  | <i>hormad1</i> | 7    | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G, A*</b> |
| 33  | <i>igf1r</i>   | 2    | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G</b>     |
| 34  | <i>igf1r</i>   | 6    | A/G          | -          | A            | A        | A, G         |
| 35  | <i>igf1r</i>   | 6    | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 36  | <i>igf1r</i>   | 18   | G/T          | -          | <b>G</b>     | <b>G</b> | <b>T</b>     |
| 37  | <i>itpr1</i>   | 5    | C/G          | -          | C            | C        | C, G         |
| 38  | <i>itpr1</i>   | 5    | A/C          | -          | C            | C        | A, C         |
| 39  | <i>itpr1</i>   | 12   | A/G          | -          | <b>G</b>     | <b>G</b> | <b>A</b>     |
| 40  | <i>itpr1</i>   | 12   | C/T          | -          | C, T         | C, T     | T            |
| 41  | <i>itpr1</i>   | 13   | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 42  | <i>itpr1</i>   | 21   | A/G          | -          | G            | G        | A, G         |
| 43  | <i>itpr1</i>   | 21   | C/T          | -          | T            | T        | C, T         |
| 44  | <i>itpr1</i>   | 21   | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G</b>     |
| 45  | <i>itpr1</i>   | 29   | C/T          | -          | <b>T, C*</b> | -        | <b>C</b>     |
| 46  | <i>itpr1</i>   | 34   | A/C          | -          | <b>A</b>     | <b>A</b> | <b>C</b>     |
| 47  | <i>kif22</i>   | 2    | A/G          | -          | A            | A, G     | A, G         |
| 48  | <i>kif22</i>   | 5    | A/C/G        | -          | A, C         | C, G     | C, G         |
| 49  | <i>kif22</i>   | 6    | A/G          | -          | A            | A, G     | A, G         |
| 50  | <i>kif22</i>   | 6    | C/T          | -          | C            | C, T     | C, T         |
| 51  | <i>kif22</i>   | 6    | A/G          | -          | A            | A, G     | A, G         |
| 52  | <i>kmt2a</i>   | 7    | A/G          | -          | A, G         | G        | A            |
| 53  | <i>kmt2a</i>   | 10   | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 54  | <i>kmt2a</i>   | 10   | A/G          | -          | <b>G</b>     | <b>G</b> | <b>A</b>     |
| 55  | <i>kmt2a</i>   | 31   | A/G          | -          | <b>G</b>     | <b>G</b> | <b>A</b>     |
| 56  | <i>m1ap</i>    | 1    | C/T          | -          | <b>T</b>     | <b>T</b> | <b>C</b>     |
| 57  | <i>m1ap</i>    | 1    | A/G          | -          | A            | A        | A, G         |
| 58  | <i>map2k1</i>  | 2    | A/G          | -          | <b>A</b>     | <b>A</b> | <b>G</b>     |
| 59  | <i>map2k1</i>  | 3    | C/T [LR]     | -          | C            | C        | C            |
| 60  | <i>map2k1</i>  | 3    | A/C          | -          | C            | C        | A, C         |
| 61  | <i>map3k7</i>  | 17   | G/T          | -          | <b>G</b>     | <b>G</b> | <b>T, G*</b> |
| 62  | <i>mapk12</i>  | 11   | C/T          | -          | <b>T</b>     | -        | <b>C</b>     |
| 63  | <i>marf1</i>   | 2    | G/T          | -          | <b>G</b>     | <b>G</b> | <b>T</b>     |

| SNP | Gene            | Exon | Substitution |     | SNP alleles |         |       |
|-----|-----------------|------|--------------|-----|-------------|---------|-------|
|     |                 |      | nt           | aa  | LL [CZ, HR] | LL [DE] | RR    |
| 64  | <i>marf1</i>    | 3    | A/C          | -   | A           | A       | A, C  |
| 65  | <i>marf1</i>    | 4    | C/T          | -   | C           | C       | T     |
| 66  | <i>marf1</i>    | 4    | C/G          | -   | C           | C       | C, G  |
| 67  | <i>mlh1</i>     | 12   | C/G          | A/G | G           | G       | C     |
| 68  | <i>mlh1</i>     | 16   | G/T          | -   | G           | G       | T     |
| 69  | <i>mlh1</i>     | 3    | G/T          | F/V | G           | G       | G, T  |
| 70  | <i>mns1</i>     | 1    | A/G          | I/V | G           | G       | A     |
| 71  | <i>mns1</i>     | 1    | A/G          | -   | G           | G       | A     |
| 72  | <i>mns1</i>     | 7    | A/G          | -   | G           | G       | A     |
| 73  | <i>mns1</i>     | 7    | A/C          | -   | A           | A       | A, C  |
| 74  | <i>mns1</i>     | 7    | C/T          | -   | C, T        | T       | C, T  |
| 75  | <i>mr11a</i>    | 14   | A/C          | -   | C           | C       | A, C  |
| 76  | <i>mre11</i>    | 14   | C/G          | A/G | C           | C       | C, G  |
| 77  | <i>mr11a</i>    | 14   | C/T          | -   | C           | C       | C, T  |
| 78  | <i>msh2</i>     | 3    | A/G          | -   | G           | G       | A, G* |
| 79  | <i>msh2</i>     | 14   | G/T          | -   | G           | G       | T     |
| 80  | <i>nbn</i>      | 10   | A/G          | N/S | G           | G       | A, G  |
| 81  | <i>nusap1</i>   | 8    | C/T          | -   | C           | T       | C, T  |
| 82  | <i>nusap1</i>   | 8    | A/G          | -   | G           | G       | A, G  |
| 83  | <i>parn</i>     | 7    | C/T          | -   | C, T        | -       | C     |
| 84  | <i>parn</i>     | 7    | A/G          | -   | G, A*       | -       | A     |
| 85  | <i>pelp1</i>    | 4    | C/T          | -   | T           | T       | C     |
| 86  | <i>pelp1</i>    | 4    | A/G          | -   | G           | G       | A, G  |
| 87  | <i>piwil2</i>   | 12   | A/C          | -   | C           | C       | A     |
| 88  | <i>piwil2</i>   | 12   | A/G          | -   | A           | A       | G     |
| 89  | <i>piwil2</i>   | 12   | C/T          | -   | C           | C       | C, T  |
| 90  | <i>piwil2</i>   | 12   | C/G          | -   | C           | C       | G     |
| 91  | <i>piwil2</i>   | 15   | C/G          | R/T | G           | G       | C     |
| 92  | <i>piwil2</i>   | 15   | A/G          | -   | G           | G       | A     |
| 93  | <i>piwil2</i>   | 15   | C/T          | N/S | T           | T       | C     |
| 94  | <i>pkmyt1</i>   | 5    | C/T          | C/R | C           | C, T    | C, T  |
| 95  | <i>pkmyt1</i>   | 5    | A/C          | P/T | C           | C       | A, C  |
| 96  | <i>plk1</i>     | 1    | A/G          | -   | G           | G       | A, G  |
| 97  | <i>plk1</i>     | 1    | A/G          | -   | G           | G       | A, G  |
| 98  | <i>plk1</i>     | 5    | G/T          | -   | G           | G       | T     |
| 99  | <i>plk1</i>     | 5    | C/T          | -   | C           | C, T*   | T, C* |
| 100 | <i>ppp1ca</i>   | 3    | C/T          | -   | C, T        | C, T    | C, T  |
| 101 | <i>ppp1ca</i>   | 3    | A/G          | -   | A           | A       | A, G  |
| 102 | <i>ppp1ca</i>   | 3    | A/T          | -   | A, T        | A, T    | A, T  |
| 103 | <i>ppp1ca</i>   | 3    | A/G          | -   | A, G        | A, G    | A, G  |
| 104 | <i>ppp1ca</i>   | 3    | C/T          | -   | C, T        | C, T    | C, T  |
| 105 | <i>ppp2r1a</i>  | 4    | C/T          | -   | C, T        | C       | C     |
| 106 | <i>ppp2r1a</i>  | 4    | C/T          | -   | C, T        | C       | C, T  |
| 107 | <i>ppp2r1a</i>  | 4    | C/T          | -   | C, T        | C       | C, T  |
| 108 | <i>ppp3ca</i>   | 2    | G/T          | -   | T           | -       | G     |
| 109 | <i>ppp3ca</i>   | 2    | A/T          | -   | A           | -       | A, T  |
| 110 | <i>prkaca</i>   | 9    | A/T          | -   | A           | -       | T     |
| 111 | <i>rad50</i>    | 5    | G/T          | A/S | G           | G       | G, T  |
| 112 | <i>rad50</i>    | 5    | A/G          | -   | G           | G       | A     |
| 113 | <i>rad51ap1</i> | 5    | A/G          | -   | A           | A       | A, G  |
| 114 | <i>rbbp8</i>    | 6    | A/G          | -   | A           | A       | G, A* |
| 115 | <i>rps6k3</i>   | 17   | A/C          | -   | C           | C       | A     |
| 116 | <i>rps6k3</i>   | 17   | A/G          | -   | G           | G       | A, G  |
| 117 | <i>rps6k3</i>   | 17   | A/C          | -   | A           | A       | C     |
| 118 | <i>sfr1</i>     | 2    | A/G          | Q/R | A, G        | A, G    | G     |
| 119 | <i>sfr1</i>     | 2    | A/G          | N/S | G           | G       | A     |
| 120 | <i>sfr1</i>     | 2    | C/T          | -   | T           | T       | C, T  |
| 121 | <i>sfr1</i>     | 2    | A/G          | C/Y | A           | A       | G     |
| 122 | <i>sgol1</i>    | 2    | A/G          | -   | A, G        | A       | A, G  |
| 123 | <i>smc1a</i>    | 4    | A/G          | -   | G           | G       | A, G  |
| 124 | <i>smc1a</i>    | 4    | C/T          | -   | C           | C       | T, C* |
| 125 | <i>smc1a</i>    | 4    | C/T          | -   | C           | C       | T, C* |
| 126 | <i>topbp1</i>   | 5    | A/G          | -   | G           | G       | A     |
| 127 | <i>trip13</i>   | 2    | A/G          | -   | G           | G       | A     |
| 128 | <i>trip13</i>   | 2    | A/G          | -   | A           | A       | G     |
| 129 | <i>ywhaz</i>    | 1    | C/T          | -   | T           | T       | C, T* |
| 130 | <i>zfp36</i>    | 2    | A/G          | -   | G           | G       | A, G  |
| 131 | <i>zfp36</i>    | 2    | C/T          | -   | C           | C       | T, C* |

**Table S5.** Basic characteristics of 160 gametogenic genes extracted from germline transcriptomes (testes) of one *P. lessonae* (LL) and three *P. ridibundus* (RR) individuals. The coding sequence length (CDSL) is given in nucleotides (nt) and the corresponding protein sequence in amino acids (aa). For *P. ridibundus*, the GC content was calculated as the arithmetic mean of individual sequence-specific values. The minimum (MIN) and maximum (MAX) of uncorrected p distances are given as a measure of interspecific genetic divergence. The number of exons (EXN) was determined from *P. lessonae* and/or *P. ridibundus* transcriptomic sequences. Uncertain results are marked with “?”. n.a. - not applicable.

| Gene            | Parameter |           | Divergence LL-RR [%] |           |      |        |       |       |                 |      |      |      |
|-----------------|-----------|-----------|----------------------|-----------|------|--------|-------|-------|-----------------|------|------|------|
|                 | CDSL (nt) |           | CDSL (aa)            |           | EXN  | GC [%] |       |       | P               |      |      |      |
|                 | LL        | RR        | LL                   | RR        |      | LL     | RR    | Xen   | P <sub>nt</sub> | MAX  | MIN  | MAX  |
| <i>adarb1</i>   | 2154      | 2154      | 717                  | 717       | n.a. | 44.90  | 44.80 | 43.87 | 1.07            | 1.11 | 0.28 | 0.28 |
| <i>adcy9</i>    | 3924      | 3924      | 1307                 | 1307      | 10   | 48.70  | 48.80 | 50.64 | 1.15            | 1.33 | 1.00 | 1.07 |
| <i>ago2</i>     | 2595      | 2622/2595 | 864                  | 873/864   | 19   | 46.40  | 46.30 | 47.16 | 0.94            | 0.94 | 0.12 | 0.12 |
| <i>ago3</i>     | 2583      | n.a.      | 860                  | n.a.      | 19   | 47.90  | n.a.  | 48.16 | n.a.            | n.a. | n.a. | n.a. |
| <i>ago4</i>     | 2589      | 2589      | 862                  | 862       | 17   | 46.90  | 47.10 | 46.93 | 0.46            | 0.50 | 0.12 | 0.12 |
| <i>akt1</i>     | 1443      | 1443      | 480                  | 480       | 12   | 40.50  | 40.60 | 41.77 | 0.42            | 0.49 | 0.21 | 0.21 |
| <i>anapc13</i>  | 225       | 225       | 74                   | 74        | n.a. | 46.20  | 46.00 | 47.11 | 0.44            | 1.33 | 0.00 | 0.00 |
| <i>ar</i>       | 2334      | n.a.      | 777                  | n.a.      | n.a. | 52.90  | n.a.  | 56.91 | n.a.            | n.a. | n.a. | n.a. |
| <i>asz1</i>     | 1425      | 1425      | 474                  | 474       | 13   | 40.70  | 41.00 | 40.13 | 0.91            | 0.91 | 0.63 | 0.63 |
| <i>atr</i>      | 7947      | 7947      | 2648                 | 2648      | 48   | 43.40  | 43.40 | 42.94 | 0.71            | 0.71 | 0.45 | 0.45 |
| <i>aurka</i>    | 1245      | 1251      | 414                  | 416       | n.a. | 49.60  | 49.60 | 53.21 | 1.29            | 1.61 | 1.21 | 1.93 |
| <i>bcar1</i>    | 2856      | 2871/2856 | 951                  | 956/951   | n.a. | 47.20  | 46.70 | 44.47 | 0.87            | 0.90 | 0.43 | 0.54 |
| <i>bcar3</i>    | 2451      | 2448      | 816                  | 815       | 11   | 45.50  | 45.40 | 45.08 | 1.06            | 1.27 | 0.86 | 0.98 |
| <i>birc5</i>    | 525       | 525       | 174                  | 174       | n.a. | 55.30  | 54.90 | 49.07 | 1.71            | 1.71 | 1.15 | 1.15 |
| <i>brd2</i>     | 2343      | 2343      | 780                  | 780       | 13   | 48.00  | 48.10 | 39.45 | 0.38            | 0.51 | 0.00 | 0.00 |
| <i>btrc</i>     | 1674      | 1674      | 557                  | 557       | 12   | 45.80  | 47.80 | 46.16 | 0.48            | 0.54 | 0.00 | 0.18 |
| <i>bub1</i>     | 3321      | 3345/3348 | 1106                 | 1114/1115 | 25   | 44.60  | 44.80 | 44.32 | 1.51            | 1.57 | 1.27 | 1.36 |
| <i>bub3</i>     | 975       | 975       | 324                  | 324       | n.a. | 48.10  | 48.10 | 48.62 | 0.31            | 0.51 | 0.00 | 0.00 |
| <i>calm</i>     | 450       | 450       | 149                  | 149       | n.a. | 43.30  | 43.30 | 40.67 | 0.00            | 0.00 | 0.00 | 0.00 |
| <i>camk2g</i>   | 1554      | 1554      | 517                  | 517       | 19   | 45.50  | 45.70 | 46.09 | 0.77            | 0.90 | 0.19 | 0.19 |
| <i>ccna1</i>    | 1254      | 1254      | 417                  | 417       | n.a. | 45.90  | 46.10 | 47.54 | 1.52            | 1.68 | 2.16 | 2.40 |
| <i>ccnb1</i>    | 1194      | 1194      | 397                  | 397       | 8?   | 54.50  | 54.50 | 44.72 | 1.59            | 1.59 | 1.01 | 1.01 |
| <i>ccnb1ip1</i> | 804       | 804       | 267                  | 267       | n.a. | 44.40  | 44.50 | 44.36 | 1.12            | 1.37 | 0.00 | 0.00 |
| <i>ccnb2</i>    | 1176      | 1176      | 391                  | 391       | n.a. | 45.70  | 46.20 | 45.61 | 1.70            | 1.70 | 1.02 | 1.02 |
| <i>ccnb3</i>    | 1251      | 1248      | 416                  | 415       | 10   | 47.10  | 46.40 | 45.08 | 2.08            | 2.08 | 1.45 | 1.45 |
| <i>ccne2</i>    | 1212      | 1212      | 403                  | 403       | 10?  | 42.60  | 42.80 | 43.55 | 0.99            | 0.99 | 1.24 | 1.24 |
| <i>cdc20</i>    | 1527      | 1527      | 508                  | 508       | 10   | 52.80  | 52.70 | 44.23 | 1.83            | 1.83 | 0.79 | 0.79 |
| <i>cdc25c</i>   | 1635      | 1635      | 544                  | 544       | 16   | 44.50  | 44.40 | 44.66 | 1.10            | 1.10 | 1.29 | 1.29 |
| <i>cdk1</i>     | 909       | 909       | 302                  | 302       | n.a. | 41.70  | 41.50 | 45.61 | 0.33            | 0.44 | 0.33 | 0.33 |
| <i>cdk2</i>     | 894       | 894       | 297                  | 297       | n.a. | 48.50  | 48.20 | 46.53 | 1.01            | 1.01 | 0.34 | 0.34 |
| <i>cdk7</i>     | 1059      | 1059      | 352                  | 352       | 11   | 43.80  | 43.70 | 44.00 | 0.00            | 1.13 | 0.00 | 0.28 |
| <i>cpeb4</i>    | 2130      | 2130      | 709                  | 709       | n.a. | 48.70  | 49.00 | 47.14 | 0.47            | 0.56 | 0.14 | 0.14 |
| <i>ctcf</i>     | 2205      | 2205      | 734                  | 734       | 10   | 42.60  | 42.60 | 44.31 | 0.45            | 0.54 | 0.27 | 0.27 |
| <i>cyp26b1</i>  | 1536      | 1536      | 511                  | 511       | n.a. | 53.70  | 53.30 | 52.80 | 1.17            | 1.17 | 0.20 | 0.20 |
| <i>dazl</i>     | 849       | 849       | 282                  | 282       | 11?  | 43.90  | 43.70 | 44.60 | 0.12            | 0.12 | 0.36 | 0.36 |
| <i>ddx19</i>    | 1479      | 1479      | 492                  | 492       | n.a. | 53.10  | 52.80 | 53.89 | 1.42            | 1.56 | 0.81 | 0.81 |
| <i>ddx25</i>    | 1464      | 1467      | 487                  | 488       | n.a. | 46.80  | 45.60 | 40.69 | 2.87            | 3.21 | 2.05 | 2.46 |
| <i>ddx4</i>     | 2226      | 2226      | 741                  | 741       | n.a. | 42.40  | 42.50 | 42.08 | 1.21            | 1.21 | 0.95 | 1.08 |
| <i>dmc1</i>     | 1026      | 1026      | 341                  | 341       | 13   | 41.00  | 41.00 | 41.23 | 0.68            | 0.78 | 0.59 | 0.59 |
| <i>dmrt1</i>    | 1005      | 1005      | 334                  | 334       | n.a. | 48.60  | 47.10 | 48.17 | 1.99            | 2.09 | 2.99 | 2.99 |
| <i>dmrt2</i>    | 1566      | n.a.      | 521                  | n.a.      | n.a. | 49.60  | n.a.  | 49.46 | n.a.            | n.a. | n.a. | n.a. |
| <i>dmrt5</i>    | 1317      | n.a.      | 438                  | n.a.      | n.a. | 58.10  | n.a.  | 55.18 | n.a.            | n.a. | n.a. | n.a. |
| <i>dnmt1</i>    | 4470/4476 | 4476      | 1489/1491            | 1491      | 32   | 45.90  | 46.10 | 46.55 | 0.87            | 0.87 | 0.67 | 0.67 |
| <i>dnmt3a</i>   | 2682      | 2682      | 893                  | 893       | 21   | 52.80  | 52.90 | 52.91 | 1.16            | 1.16 | 0.45 | 0.45 |
| <i>dusp1</i>    | 1110      | 1110      | 369                  | 369       | n.a. | 49.70  | 49.60 | 48.56 | 0.81            | 0.99 | 0.00 | 0.00 |
| <i>elf4g3</i>   | 5160      | 5160      | 1719                 | 1719      | n.a. | 57.00  | 56.90 | 57.48 | 1.32            | 1.45 | 0.99 | 1.05 |
| <i>eme1</i>     | 777       | 777       | 258                  | 258       | n.a. | 49.80  | 49.30 | 55.53 | 1.80            | 1.80 | 3.49 | 3.49 |
| <i>eme2</i>     | 1560      | 1560      | 519                  | 519       | n.a. | 47.90  | 47.90 | 49.54 | 1.73            | 2.12 | 2.51 | 2.89 |
| <i>exd1</i>     | 1155      | 1155      | 384                  | 384       | 10   | 42.30  | 42.30 | 44.16 | 0.00            | 0.00 | 0.00 | 0.00 |
| <i>fbxo43</i>   | 2157      | 2160      | 718                  | 719       | n.a. | 42.20  | 41.70 | 43.06 | 1.72            | 1.76 | 2.24 | 2.37 |
| <i>fbxo5</i>    | 1212      | 1170      | 403                  | 389       | n.a. | 41.60  | 41.50 | 40.56 | 1.45            | 1.97 | 1.55 | 2.32 |
| <i>fgf9</i>     | 630       | 630       | 209                  | 209       | n.a. | 46.60  | 46.60 | 48.80 | 0.16            | 0.48 | 0.00 | 0.00 |



| Gene           | Parameter |           |           |         | EXN  | Divergence LL-RR [%] |       |       |                 |      |                 |      |  |
|----------------|-----------|-----------|-----------|---------|------|----------------------|-------|-------|-----------------|------|-----------------|------|--|
|                | CDSL (nt) |           | CDSL (aa) |         |      | GC [%]               |       |       | P <sub>nt</sub> |      | P <sub>aa</sub> |      |  |
|                | LL        | RR        | LL        | RR      |      | LL                   | RR    | Xen   | MIN             | MAX  | MIN             | MAX  |  |
| <i>fkbp6</i>   | 990       | 990       | 329       | 329     | n.a. | 45.00                | 45.40 | 45.58 | 1.16            | 1.57 | 1.06            | 1.37 |  |
| <i>foxc1</i>   | 1482      | 1488      | 493       | 495     | 1?   | 54.90                | 54.60 | 59.61 | 0.88            | 1.01 | 0.41            | 0.61 |  |
| <i>grb2</i>    | 690       | 690       | 229       | 229     | n.a. | 46.60                | 47.20 | 49.86 | 1.45            | 1.59 | 0.00            | 0.00 |  |
| <i>h3.3</i>    | 411       | 411       | 136       | 136     | n.a. | 56.00                | 56.20 | 55.23 | 0.24            | 0.24 | 0.00            | 0.00 |  |
| <i>hat1</i>    | 1227      | 1227      | 408       | 408     | 11   | 42.80                | 42.40 | 42.35 | 0.73            | 0.73 | 0.74            | 0.74 |  |
| <i>hdac1</i>   | 1443      | 1443      | 480       | 480     | 14   | 42.80                | 42.90 | 42.97 | 0.62            | 0.69 | 0.00            | 0.00 |  |
| <i>hec1</i>    | 1917      | 1917      | 638       | 638     | 16   | 51.80                | 51.70 | 53.15 | 1.15            | 1.57 | 0.63            | 1.41 |  |
| <i>henmt1</i>  | 1125      | 1125      | 374       | 374     | n.a. | 39.70                | 39.50 | 42.61 | 0.80            | 1.16 | 1.60            | 1.87 |  |
| <i>hormad1</i> | 1161      | 1161      | 386       | 386     | 12   | 44.40                | 44.60 | 43.90 | 0.86            | 1.03 | 1.04            | 1.04 |  |
| <i>igf1</i>    | 462       | 462       | 153       | 153     | n.a. | 45.40                | 44.70 | 45.45 | 1.08            | 1.08 | 0.65            | 0.65 |  |
| <i>igf1r</i>   | 3330      | 3327      | 1109      | 1108    | 18   | 43.50                | 44.60 | 47.84 | 0.64            | 0.64 | 0.83            | 0.83 |  |
| <i>ints13</i>  | 2157      | 2157      | 718       | 718     | 17   | 48.60                | 48.50 | 56.17 | 0.37            | 1.39 | 0.14            | 0.98 |  |
| <i>itpr1</i>   | 8049      | 8043      | 2682      | 2680    | 47?  | 52.50                | 52.70 | 46.49 | 1.50            | 1.53 | 0.37            | 0.37 |  |
| <i>kif22</i>   | 1929      | 1929      | 642       | 642     | 13   | 49.70                | 49.60 | 57.85 | 0.52            | 0.83 | 0.31            | 0.47 |  |
| <i>kmt2a</i>   | 7983      | n.a.      | 2660      | n.a.    | 31   | 45.50                | 45.60 | 46.00 | 0.89            | 0.89 | 0.96            | 0.96 |  |
| <i>m1ap</i>    | 1461      | 1464      | 486       | 487     | n.a. | 56.20                | 56.30 | 54.93 | 2.26            | 2.26 | 2.06            | 2.06 |  |
| <i>mad2l1</i>  | 612       | 612       | 203       | 203     | n.a. | 44.50                | 44.30 | 40.85 | 0.65            | 0.65 | 0.49            | 0.49 |  |
| <i>mael</i>    | 1266      | 1263      | 421       | 420     | 13   | 46.00                | 46.40 | 45.91 | 2.69            | 2.69 | 3.10            | 3.10 |  |
| <i>map2k1</i>  | 1227      | 1227      | 408       | 408     | 11   | 51.90                | 51.70 | 48.40 | 0.73            | 0.90 | 0.00            | 0.00 |  |
| <i>map3k7</i>  | 1827      | 1827      | 608       | 608     | 17   | 44.00                | 44.10 | 42.82 | 0.38            | 0.44 | 0.33            | 0.33 |  |
| <i>mapk1</i>   | 1089      | 1089      | 362       | 362     | n.a. | 43.80                | 43.90 | 43.74 | 0.37            | 0.46 | 0.00            | 0.00 |  |
| <i>mapk12</i>  | 1089      | 1089      | 362       | 362     | 12   | 44.60                | 44.50 | 42.14 | 0.83            | 0.92 | 0.28            | 0.28 |  |
| <i>marf1</i>   | 5064      | 5064      | 1687      | 1687    | 25   | 43.50                | 43.70 | 43.32 | 1.26            | 1.32 | 1.36            | 1.54 |  |
| <i>mdc1</i>    | 6771      | 6708      | 2256      | 2235    | 16   | 45.80                | 45.70 | 41.53 | 2.91            | 2.95 | 5.33            | 5.42 |  |
| <i>mei4</i>    | 1212      | n.a.      | 403       | n.a.    | n.a. | 36.80                | 38.20 | 36.39 | 1.38            | 1.38 | 2.07            | 2.07 |  |
| <i>meig1</i>   | 276       | 276       | 91        | 91      | n.a. | 38.70                | 38.40 | 35.96 | 0.36            | 0.36 | 0.00            | 0.00 |  |
| <i>meiob</i>   | 1419      | 1419      | 472       | 472     | 13   | 43.70                | 43.70 | 42.50 | 1.34            | 1.34 | 1.06            | 1.06 |  |
| <i>mlh1</i>    | 2265      | 2265      | 754       | 754     | 19   | 42.40                | 42.20 | 41.56 | 1.19            | 1.46 | 0.93            | 1.19 |  |
| <i>mlh3</i>    | 3681      | 3708      | 1226      | 1235    | 12   | 49.00                | 48.60 | 39.34 | 2.43            | 2.43 | 4.15            | 4.32 |  |
| <i>mnd1</i>    | 618       | 618       | 205       | 205     | n.a. | 43.40                | 42.90 | 41.19 | 0.16            | 1.78 | 0.00            | 2.44 |  |
| <i>mns1</i>    | 1500      | 1500      | 499       | 499     | n.a. | 43.60                | 43.30 | 42.02 | 1.07            | 1.13 | 0.80            | 1.00 |  |
| <i>mov10l1</i> | 3690      | 3687      | 1229      | 1228    | 26   | 41.30                | 41.20 | 42.09 | 1.00            | 1.00 | 1.06            | 1.06 |  |
| <i>mre11</i>   | 2136      | 2136      | 711       | 711     | 19   | 45.20                | 44.80 | 46.95 | 1.26            | 1.40 | 0.42            | 0.84 |  |
| <i>msh2</i>    | 2793      | 2793      | 930       | 930     | 16   | 47.20                | 47.10 | 44.98 | 1.33            | 1.33 | 0.43            | 0.43 |  |
| <i>msh4</i>    | 2289      | 2289      | 762       | 762     | 18   | 41.50                | 41.40 | 37.46 | 1.32            | 1.59 | 0.66            | 0.80 |  |
| <i>msh5</i>    | 2424      | 2424      | 807       | 807     | 25   | 43.80                | 43.70 | 42.68 | 0.87            | 0.95 | 0.74            | 0.74 |  |
| <i>msh6</i>    | 4017      | 4017      | 1338      | 1338    | 10   | 46.20                | 46.20 | 45.36 | 1.22            | 1.22 | 0.90            | 0.97 |  |
| <i>msx1</i>    | 843       | 843       | 280       | 280     | n.a. | 50.80                | 50.70 | 57.00 | 0.71            | 0.83 | 1.07            | 1.07 |  |
| <i>mus81</i>   | 1818      | 1818      | 605       | 605     | 16   | 44.00                | 43.90 | 46.33 | 0.99            | 0.99 | 1.33            | 1.82 |  |
| <i>mybl1</i>   | 804       | 804       | 267       | 267     | n.a. | 44.60                | 44.50 | 43.05 | 0.00            | 0.25 | 0.00            | 0.00 |  |
| <i>nbn</i>     | 2301      | 2292/2289 | 766       | 763/762 | 16   | 41.50                | 41.80 | 42.53 | 1.53            | 1.66 | 2.23            | 2.49 |  |
| <i>nusap1</i>  | 1263      | 1263      | 420       | 420     | 11   | 41.60                | 42.10 | 46.20 | 0.00            | 0.08 | 0.00            | 0.24 |  |
| <i>parn</i>    | 1905      | 1905      | 634       | 634     | 24   | 40.10                | 40.20 | 40.18 | 1.68            | 1.79 | 1.42            | 1.74 |  |
| <i>pelp1</i>   | 3270      | 3270      | 1089      | 1089    | 16   | 52.20                | 52.40 | 57.80 | 1.13            | 1.26 | 0.83            | 1.01 |  |
| <i>piwil2</i>  | 2853      | 2853      | 950       | 950     | 24   | 46.10                | 46.10 | 46.42 | 1.54            | 1.68 | 1.68            | 1.90 |  |
| <i>piwil4</i>  | 2502      | 2502      | 833       | 833     | 20   | 45.90                | 45.80 | 50.78 | 0.64            | 0.76 | 0.60            | 0.72 |  |
| <i>pkmyt1</i>  | 1644      | 1644      | 547       | 547     | n.a. | 56.20                | 56.10 | 50.91 | 1.22            | 2.07 | 0.91            | 1.83 |  |
| <i>pld6</i>    | 492       | 492       | 163       | 163     | n.a. | 55.60                | 55.40 | 56.71 | 1.02            | 1.42 | 0.00            | 0.61 |  |
| <i>plk1</i>    | 1779      | 1779      | 592       | 592     | 10   | 51.30                | 50.90 | 50.08 | 1.57            | 1.57 | 0.68            | 0.68 |  |
| <i>pms1</i>    | 2664      | 2664      | 887       | 887     | 12   | 38.60                | 38.90 | 37.31 | 1.80            | 1.84 | 3.04            | 3.27 |  |
| <i>pms2</i>    | 2517      | 2517/2520 | 838       | 838/839 | 13   | 40.00                | 39.80 | 44.64 | 0.95            | 1.07 | 1.43            | 1.67 |  |
| <i>ppp1ca</i>  | 984       | 984       | 327       | 327     | n.a. | 48.90                | 48.80 | 57.19 | 0.00            | 1.52 | 0.00            | 0.00 |  |
| <i>ppp2r1a</i> | 1770      | 1770      | 589       | 589     | 15   | 52.20                | 51.90 | 49.44 | 1.64            | 1.75 | 0.17            | 0.17 |  |
| <i>ppp2r5a</i> | 1422      | 1422      | 473       | 473     | 13   | 43.80                | 43.90 | 43.33 | 0.84            | 0.91 | 0.21            | 0.21 |  |
| <i>ppp3ca</i>  | 1533      | 1533      | 510       | 510     | 13   | 43.30                | 43.40 | 41.08 | 0.72            | 0.78 | 0.20            | 0.20 |  |
| <i>prc1</i>    | 1827      | 1827      | 608       | 608     | 14   | 42.50                | 42.90 | 41.98 | 0.88            | 0.88 | 0.16            | 0.16 |  |
| <i>prdm9</i>   | 1854      | 1854      | 617       | 617     | 1?   | 53.20                | 53.00 | 43.95 | 1.29            | 1.67 | 1.30            | 2.11 |  |
| <i>prkaca</i>  | 1056      | 1056      | 351       | 351     | 10   | 44.10                | 43.80 | 46.78 | 0.47            | 0.57 | 0.00            | 0.00 |  |
| <i>psmc3ip</i> | 663       | 645       | 220       | 214     | n.a. | 43.50                | 42.90 | 41.67 | 2.33            | 2.33 | 1.87            | 2.34 |  |
| <i>pttg1</i>   | 564       | 564       | 187       | 187     | n.a. | 44.30                | 44.30 | 39.49 | 1.42            | 1.60 | 2.67            | 3.21 |  |
| <i>rad21</i>   | 1890      | 1887      | 629       | 628     | 13   | 47.90                | 48.00 | 48.15 | 0.80            | 0.90 | 0.32            | 0.64 |  |

| Gene     | Parameter |           |           |           | EXN  | Divergence LL-RR [%] |       |       |                 |      |                 |      |
|----------|-----------|-----------|-----------|-----------|------|----------------------|-------|-------|-----------------|------|-----------------|------|
|          | CDSL (nt) |           | CDSL (aa) |           |      | GC [%]               |       |       | P <sub>nt</sub> |      | P <sub>aa</sub> |      |
|          | LL        | RR        | LL        | RR        |      | LL                   | RR    | Xen   | MIN             | MAX  | MIN             | MAX  |
| rad50    | 3939      | 3939      | 1312      | 1312      | 25   | 43.70                | 44.40 | 41.53 | 0.86            | 0.89 | 0.61            | 0.61 |
| rad51    | 1008      | 1008      | 335       | 335       | n.a. | 45.10                | 45.10 | 49.26 | 0.00            | 0.00 | 0.00            | 0.00 |
| rad51ap1 | 1098      | 1101      | 365       | 366       | n.a. | 44.30                | 44.80 | 44.93 | 1.91            | 2.00 | 1.64            | 1.92 |
| rad52    | 1302      | 1290      | 433       | 429       | 11   | 50.00                | 50.00 | 54.80 | 0.16            | 1.01 | 0.47            | 1.17 |
| rad54l   | 2229      | 2229      | 742       | 742       | 18   | 55.20                | 55.50 | 44.24 | 2.02            | 2.15 | 0.81            | 0.81 |
| rbbp8    | 2607      | 2610      | 868       | 869       | 17   | 43.70                | 43.70 | 43.91 | 1.14            | 1.14 | 1.38            | 1.38 |
| rec114   | 651       | n.a.      | 216       | n.a.      | n.a. | 44.10                | 44.00 | n.a.  | 0.77            | 0.77 | 0.93            | 0.93 |
| rec8     | 2277      | 2277      | 758       | 758       | 19   | 46.30                | 46.80 | 48.03 | 1.45            | 1.45 | 1.98            | 1.98 |
| recq1    | 1974      | 1974      | 657       | 657       | 15   | 47.40                | 47.40 | 57.84 | 0.91            | 1.11 | 0.46            | 0.76 |
| rpa1     | 1839      | 1839      | 612       | 612       | 17   | 44.70                | 44.90 | 43.44 | 1.09            | 1.14 | 0.65            | 0.65 |
| rpa2     | 831       | 831       | 276       | 276       | n.a. | 44.30                | 44.80 | 45.41 | 0.60            | 0.84 | 0.00            | 0.00 |
| rpa3     | 366       | 366       | 121       | 121       | n.a. | 48.40                | 47.90 | 44.26 | 0.27            | 3.01 | 0.00            | 2.48 |
| rps6ka3  | 2214      | 2214      | 737       | 737       | 22   | 43.70                | 43.90 | 43.34 | 1.08            | 1.13 | 0.27            | 0.27 |
| sfr1     | 729       | 714       | 242       | 237       | n.a. | 44.60                | 45.20 | 40.29 | 1.96            | 1.96 | 2.95            | 2.95 |
| sgo1     | 2016      | 2019      | 671       | 672       | n.a. | 41.60                | 41.40 | 40.87 | 1.75            | 1.75 | 2.64            | 2.64 |
| skp1     | 492       | 492       | 163       | 163       | n.a. | 39.90                | 40.10 | 40.24 | 0.41            | 0.41 | 0.00            | 0.00 |
| smc1a    | 3687      | 3687      | 1228      | 1228      | 25   | 49.20                | 48.90 | 46.23 | 1.22            | 1.25 | 0.00            | 0.08 |
| smc1b    | 3687      | 3687      | 1228      | 1228      | 25   | 40.80                | 40.60 | 46.51 | 0.49            | 0.54 | 0.24            | 0.41 |
| smc3     | 3654      | 3654      | 1217      | 1217      | n.a. | 44.70                | 45.10 | 46.66 | 0.85            | 0.85 | 0.08            | 0.08 |
| spata1   | 1497      | n.a.      | 498       | n.a.      | 12   | 46.70                | n.a.  | 40.58 | n.a.            | n.a. | n.a.            | n.a. |
| spata2   | 1575      | 1575      | 524       | 524       | n.a. | 49.70                | 50.00 | 45.96 | 1.53            | 1.59 | 0.95            | 0.95 |
| spata22  | 957       | 957       | 318       | 318       | n.a. | 39.20                | 38.50 | 38.76 | 1.46            | 1.57 | 2.83            | 3.15 |
| spata4   | 816       | 816       | 271       | 271       | n.a. | 43.20                | 42.80 | 44.73 | 0.98            | 1.10 | 0.00            | 0.00 |
| spata5   | 2565      | 2565      | 854       | 854       | 17   | 45.40                | 45.60 | 45.28 | 1.48            | 1.48 | 1.41            | 1.41 |
| spdyc    | 876       | 876       | 291       | 291       | n.a. | 49.10                | 48.80 | 47.10 | 0.57            | 0.69 | 0.34            | 0.34 |
| spo11    | 1206      | 1206      | 401       | 401       | 13   | 49.20                | 48.70 | 45.81 | 1.24            | 1.74 | 1.25            | 1.50 |
| stra8    | 807       | 807       | 268       | 268       | n.a. | 48.80                | 49.10 | 52.78 | 1.12            | 1.12 | 1.87            | 1.87 |
| sumo1    | 309       | 309       | 102       | 102       | n.a. | 42.40                | 42.70 | 42.07 | 0.65            | 0.65 | 0.98            | 0.98 |
| sun1     | 2733      | 2727      | 910       | 908       | n.a. | 44.30                | 44.00 | 44.10 | 1.03            | 1.06 | 1.10            | 1.21 |
| suv39h1  | 1275      | 1275      | 424       | 424       | n.a. | 46.80                | 46.90 | 60.38 | 0.63            | 0.63 | 0.94            | 0.94 |
| syce2    | 507       | 507       | 168       | 168       | n.a. | 46.80                | 47.10 | 46.27 | 1.58            | 1.78 | 2.38            | 2.98 |
| syce3    | 267       | 267       | 88        | 88        | n.a. | 44.20                | 44.50 | 43.88 | 0.00            | 0.75 | 0.00            | 0.00 |
| sycp1    | 2994      | 2994      | 997       | 997       | 30   | 34.90                | 34.80 | 34.81 | 0.77            | 1.00 | 1.20            | 2.01 |
| sycp3    | 717       | 717       | 238       | 238       | n.a. | 39.00                | 38.50 | 41.38 | 0.84            | 0.84 | 1.26            | 1.26 |
| tdrd1    | 4014      | 4017/4005 | 1337      | 1338/1334 | 22   | 44.90                | 45.10 | 50.81 | 0.58            | 1.19 | 1.71            | 2.26 |
| tdrd3    | 2232      | 2232      | 743       | 743       | 13   | 42.7                 | 42.50 | 41.96 | 1.21            | 1.34 | 1.48            | 1.62 |
| tdrd5    | 3249      | 3249      | 1082      | 1082      | 16   | 43.80                | 43.70 | 41.91 | 0.62            | 0.62 | 1.20            | 1.20 |
| tdrd9    | 4038      | 4038      | 1345      | 1345      | 36   | 43.00                | 43.10 | 38.66 | 0.84            | 0.84 | 0.97            | 0.97 |
| tex11    | 2763      | 2763      | 920       | 920       | 29   | 50.00                | 49.50 | 43.19 | 2.03            | 2.03 | 2.94            | 2.94 |
| tex12    | 354       | 354       | 117       | 117       | n.a. | 38.70                | 39.60 | n.a.  | 1.41            | 1.41 | 2.56            | 2.56 |
| tex14    | 3369      | 3381      | 1122      | 1126      | 26   | 40.10                | 40.00 | 37.66 | 1.10            | 1.10 | 1.87            | 1.87 |
| topbp1   | 4548      | 4548      | 1515      | 1515      | 27   | 44.10                | 44.00 | 43.34 | 0.99            | 0.99 | 0.92            | 0.99 |
| trip13   | 1299      | 1299      | 432       | 432       | 13   | 38.40                | 38.50 | 38.17 | 1.31            | 1.31 | 0.93            | 0.93 |
| ttk      | 2628      | 2622      | 875       | 873       | 18   | 40.50                | 40.60 | 40.61 | 1.76            | 1.76 | 2.41            | 2.41 |
| wee1     | 1731      | 1731      | 576       | 576       | 11   | 56.40                | 56.00 | 56.64 | 1.10            | 1.73 | 0.52            | 0.69 |
| ywhaz    | 741       | 741       | 246       | 246       | n.a. | 46.90                | 47.10 | 45.93 | 0.41            | 0.41 | 0.00            | 0.00 |
| zfp36    | 1134      | 1131      | 377       | 376       | n.a. | 58.40                | 57.60 | 50.11 | 1.42            | 1.59 | 0.27            | 0.80 |

**Table S6.** Best-fit models and statistical parameters of simple regressions performed with the number of exons (EXN), coding sequence length (CDSL), and GC content (GC) of gametogenic genes obtained from the transcriptomes of *P. lessonae* (LL) and *P. ridibundus* (RR). The Durbin-Watson statistic (DWS) was used to assess serial correlation in the residuals; a value close to 2 indicates that the residuals vary randomly. Significant probability values (*p*) are highlighted in bold. N: sample size.

| Species | N   | Y   | X    | Model                                             | R <sup>2</sup> [%] | ANOVA  |          | DWS ( <i>p</i> )      |
|---------|-----|-----|------|---------------------------------------------------|--------------------|--------|----------|-----------------------|
|         |     |     |      |                                                   |                    | F      | <i>p</i> |                       |
| LL      | 156 | EXN | CDSL | $Y = -7.32 + 0.475 \cdot \sqrt{X}$                | 64.7               | 282.55 | <0.001   | 1.82 (0.134)          |
|         | 160 | GC  | CDSL | $Y = 1/(0.0218 + 0.193 \div X)$                   | 0.4                | 0.64   | 0.422    | 1.72 ( <b>0.040</b> ) |
|         | 156 | GC  | EXN  | $Y = \sqrt{(2.0 \cdot 10^3 + 889 \div X)}$        | 9.9                | 16.94  | <0.001   | 1.87 (0.213)          |
| RR      | 149 | EXN | CDSL | $Y = \sqrt{(61.7 + 3.0 \cdot 10^{-5} \cdot X^2)}$ | 68.2               | 314.86 | <0.001   | 2.02 (0.538)          |
|         | 153 | GC  | CDSL | $Y = 1/(2.2 \cdot 10^{-2} + 0.187 \div X)$        | 0.06               | 0.62   | 0.432    | 1.77 (0.074)          |
|         | 152 | GC  | EXN  | $Y = \sqrt{(2.02 \cdot 10^3 + 750 \div X)}$       | 7.4                | 11.86  | <0.001   | 1.88 (0.226)          |

**Table S7.** Best-fit models (simple linear regressions) for the relationships between the dependent parameter genetic distance (*p<sub>nt</sub>*), based on nucleotide sequences of gametogenic genes, and the independent parameters GC content, coding sequence length (CDSL), and number of exons (EXN). Regression analyses were conducted for both interspecific comparisons (*P. lessonae* [LL] vs. *P. ridibundus* [RR]) and intraspecific comparisons among RR individuals (1–3). The best-fitting model for each relationship was selected based on the highest R<sup>2</sup> value. The Durbin-Watson statistic (DWS) was used to assess serial correlation in the residuals; a value close to 2 indicates that the residuals vary randomly. Significant probability values (*p*) are highlighted in bold. N: sample size.

| Comparison | X                    | N   | Best fit model                                                      | R <sup>2</sup> [%] | ANOVA |              | DWS ( <i>p</i> ) |
|------------|----------------------|-----|---------------------------------------------------------------------|--------------------|-------|--------------|------------------|
|            |                      |     |                                                                     |                    | F     | <i>p</i>     |                  |
| LL-RR      | GC <sub>mean</sub>   | 155 | $p_{nt} = 4.55 \cdot 10^{-3} + 3.3 \cdot 10^{-6} \cdot X^2$         | 5.70               | 9.25  | <b>0.003</b> | 2.03 (0.568)     |
|            | CDSL <sub>mean</sub> | 152 | $p_{nt} = (0.038 + 8.79 \cdot 10^{-3} \cdot \ln(X))^2$              | 4.02               | 6.29  | <b>0.013</b> | 2.04 (0.597)     |
|            | EXN                  | 151 | $p_{nt} = \sqrt{(1.66 \cdot 10^{-4} - 2.35 \cdot 10^{-8} \cdot X)}$ | 0.25               | 0.37  | 0.543        | 2.13 (0.780)     |
| RR (1-3)   | GC                   | 148 | $p_{nt} = -4.06 \cdot 10^{-3} + 1.36 \cdot 10^{-4} \cdot X$         | 3.94               | 5.98  | <b>0.016</b> | 2.19 (0.880)     |
|            | CDSL                 | 148 | $p_{nt} = \sqrt{(5.80 \cdot 10^{-6} + 9.78 \cdot 10^{-3} \div X)}$  | 3.15               | 4.75  | <b>0.031</b> | 2.16 (0.841)     |
|            | EXN                  | 144 | $p_{nt} = 1.7 \cdot 10^{-3} + 3.45 \cdot 10^{-3} \div X$            | 2.87               | 4.19  | <b>0.042</b> | 2.22 (0.910)     |

**Table S8.** Medians ( $\bar{x}$ ) of SNP frequencies for *Pelophylax ridibundus* (RR) and diploid *Pelophylax esculentus* (LR). Mann-Whitney-(Wilcoxon) (MWW), Kruskal-Wallis and Mood's median tests were used to test for  $\bar{x}$  differences between population systems. Significant differences are highlighted in bold. R: all-*ridibundus* population, R-E: *ridibundus-esculentus* population, L-E: *lessonae-esculentus* population, E: all-hybrid (*esculentus*) population. n.a.: not applicable due to insufficient data.

| SNP | RR        |       |      |       | LR        |       |       |                |              |                |              |
|-----|-----------|-------|------|-------|-----------|-------|-------|----------------|--------------|----------------|--------------|
|     | $\bar{x}$ |       | MWW  |       | $\bar{x}$ |       |       | Kruskal-Wallis |              | Mood's Median  |              |
|     | R         | R-E   | W    | p     | L-E       | E     | R-E   | H              | p            | X <sup>2</sup> | p            |
| 1   | 0.000     | 0.000 | 9.0  | 0.136 | 0.500     | 0.500 | 0.550 | 1.783          | 0.410        | 2.100          | 0.350        |
| 2   | 0.000     | 0.000 | 9.0  | 0.136 | 0.500     | 0.500 | 0.550 | 1.783          | 0.410        | 2.100          | 0.350        |
| 3   | 0.130     | 0.170 | 5.0  | 0.141 | 0.400     | 0.130 | 0.090 | 1.056          | 0.590        | 1.533          | 0.465        |
| 4   | 0.790     | 0.700 | 13.5 | 0.459 | 0.630     | 0.625 | 0.865 | 1.384          | 0.501        | 2.261          | 0.323        |
| 5   | 0.925     | 0.800 | 25.0 | 0.082 | 0.630     | 0.890 | 0.875 | 0.891          | 0.640        | 0.533          | 0.766        |
| 6   | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 7   | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 8   | 0.170     | 0.060 | 18.0 | 0.287 | 0.500     | 0.500 | 0.520 | 1.590          | 0.452        | 2.177          | 0.337        |
| 9   | 0.830     | 0.500 | 13.0 | 1.000 | 0.900     | 1.000 | 0.855 | 1.600          | 0.449        | 3.133          | 0.209        |
| 10  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 11  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 12  | 1.000     | 1.000 | 12.5 | 0.465 | 1.000     | 1.000 | 1.000 | —              | —            | —              | —            |
| 13  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.400          | 0.497        | 1.527          | 0.466        |
| 14  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 15  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 16  | 0.250     | 0.080 | 16.5 | 0.458 | 0.500     | 0.500 | 0.585 | 2.684          | 0.261        | 2.400          | 0.301        |
| 17  | 0.130     | 0.000 | 16.0 | 0.921 | 0.500     | 0.620 | 0.585 | 3.257          | 0.196        | 4.800          | 0.091        |
| 18  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.750          | 0.417        | n.a.           | n.a.         |
| 19  | 0.500     | 0.670 | 8.5  | 0.458 | 0.380     | 0.025 | 0.270 | 0.586          | 0.746        | 1.253          | 0.535        |
| 20  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 21  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 22  | 1.000     | 1.000 | 12.5 | 0.465 | 1.000     | 1.000 | 1.000 | —              | —            | —              | —            |
| 23  | 1.000     | 1.000 | —    | —     | 0.750     | 0.500 | 0.500 | <b>9.128</b>   | <b>0.010</b> | <b>12.000</b>  | <b>0.002</b> |
| 24  | 0.000     | 0.000 | 17.5 | 0.465 | 0.500     | 0.500 | 0.550 | <b>7.157</b>   | <b>0.028</b> | <b>8.000</b>   | <b>0.018</b> |
| 25  | 0.000     | 0.000 | 12.0 | 0.361 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 26  | 0.780     | 0.440 | 22.5 | 0.200 | 0.400     | 0.250 | 0.165 | 1.765          | 0.414        | 1.234          | 0.540        |
| 27  | 0.550     | 0.440 | 19.0 | 0.523 | 0.400     | 0.250 | 0.165 | 1.765          | 0.414        | 1.234          | 0.540        |
| 28  | 0.460     | 0.180 | 23.0 | 0.170 | 0.200     | 0.000 | 0.030 | <b>8.824</b>   | <b>0.012</b> | <b>7.886</b>   | <b>0.019</b> |
| 29  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 30  | 0.000     | 0.000 | 9.0  | 0.134 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 31  | 1.000     | 1.000 | 9.0  | 0.134 | 0.880     | 0.500 | 0.500 | <b>8.437</b>   | <b>0.015</b> | <b>7.063</b>   | <b>0.029</b> |
| 32  | 0.000     | 0.000 | 9.0  | 0.134 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 33  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 34  | 0.945     | 0.830 | 20.0 | 0.409 | 1.000     | 1.000 | 0.900 | 5.189          | 0.075        | n.a.           | n.a.         |
| 35  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 36  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 37  | 0.950     | 0.890 | 18.0 | 0.647 | 1.000     | 1.000 | 0.940 | 1.830          | 0.401        | n.a.           | n.a.         |
| 38  | 0.950     | 0.890 | 18.0 | 0.647 | 0.500     | 0.500 | 0.440 | 1.830          | 0.401        | n.a.           | n.a.         |
| 39  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 40  | 0.000     | 0.000 | —    | —     | 0.000     | 0.150 | 0.000 | 3.790          | 0.150        | 3.600          | 0.165        |
| 41  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 42  | 0.030     | 0.070 | 16.0 | 0.925 | 0.000     | 0.000 | 0.025 | 2.611          | 0.271        | 3.111          | 0.211        |
| 43  | 0.140     | 0.210 | 8.5  | 0.271 | 0.000     | 0.000 | 0.120 | 2.000          | 0.367        | 6.133          | 0.047        |
| 44  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | 3.000          | 0.223        | n.a.           | n.a.         |
| 45  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 3.000          | 0.223        | 3.273          | 0.195        |
| 46  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 47  | 0.030     | 0.070 | 13.0 | 0.779 | 0.500     | 0.000 | 0.020 | <b>7.484</b>   | <b>0.024</b> | 4.800          | 0.091        |
| 48  | 0.325     | 0.210 | 20.5 | 0.360 | 0.500     | 0.270 | 0.145 | <b>6.483</b>   | <b>0.039</b> | <b>7.200</b>   | <b>0.027</b> |
| 49  | 0.300     | 0.210 | 19.5 | 0.464 | 0.500     | 0.250 | 0.160 | <b>6.414</b>   | <b>0.040</b> | <b>7.200</b>   | <b>0.027</b> |
| 50  | 0.765     | 0.850 | 10.5 | 0.464 | 1.000     | 0.750 | 0.905 | 1.864          | 0.394        | 0.533          | 0.766        |
| 51  | 0.700     | 0.790 | 10.5 | 0.464 | 0.500     | 0.750 | 0.840 | <b>6.414</b>   | <b>0.040</b> | <b>7.200</b>   | <b>0.027</b> |
| 52  | 1.000     | 1.000 | —    | —     | 0.750     | 0.500 | 0.500 | <b>7.493</b>   | <b>0.024</b> | <b>7.063</b>   | <b>0.029</b> |
| 53  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 54  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 55  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 56  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 57  | 0.000     | 0.000 | 15.0 | 0.916 | 0.500     | 0.500 | 0.525 | 4.716          | 0.095        | 4.800          | 0.091        |
| 58  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 59  | 1.000     | 1.000 | —    | —     | 1.000     | 1.000 | 1.000 | 3.000          | 0.223        | n.a.           | n.a.         |
| 60  | 0.580     | 0.530 | 22.0 | 0.231 | 0.000     | 0.100 | 0.220 | 3.713          | 0.156        | 3.977          | 0.137        |

| SNP | RR        |       |      |       | LR        |       |       |                |              |                |              |
|-----|-----------|-------|------|-------|-----------|-------|-------|----------------|--------------|----------------|--------------|
|     | $\bar{x}$ |       | MWW  |       | $\bar{x}$ |       |       | Kruskal-Wallis |              | Mood's Median  |              |
|     | R         | R-E   | W    | p     | L-E       | E     | R-E   | H              | p            | X <sup>2</sup> | p            |
| 61  | 0.000     | 0.000 | 17.5 | 0.465 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 62  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 63  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 64  | 0.450     | 0.140 | 21.0 | 0.315 | 1.000     | 0.670 | 0.740 | 4.629          | 0.099        | 4.800          | 0.091        |
| 65  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 66  | 0.140     | 0.120 | 16.5 | 0.855 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 67  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.431          | 0.489        | n.a.           | n.a.         |
| 68  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 69  | 0.230     | 0.310 | 17.0 | 0.784 | 0.500     | 0.760 | 0.575 | 1.871          | 0.393        | 0.533          | 0.766        |
| 70  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.750          | 0.417        | 1.925          | 0.382        |
| 71  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.750          | 0.417        | 1.925          | 0.382        |
| 72  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 73  | 0.290     | 0.190 | 16.5 | 0.855 | 0.500     | 0.900 | 0.770 | 2.543          | 0.280        | 0.533          | 0.766        |
| 74  | 0.880     | 0.850 | 14.0 | 0.926 | 0.830     | 0.500 | 0.380 | <b>7.381</b>   | <b>0.025</b> | <b>7.200</b>   | <b>0.027</b> |
| 75  | 0.765     | 0.910 | 7.5  | 0.200 | 0.500     | 0.390 | 0.415 | 4.069          | 0.131        | 4.200          | 0.123        |
| 76  | 0.570     | 0.270 | 24.0 | 0.121 | 0.500     | 0.610 | 0.665 | 2.311          | 0.315        | 1.533          | 0.465        |
| 77  | 1.000     | 1.000 | 12.5 | 0.465 | 1.000     | 1.000 | 1.000 | —              | —            | —              | —            |
| 78  | 1.000     | 1.000 | 12.5 | 0.465 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 79  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 80  | 0.725     | 0.730 | 15.5 | 1.000 | 0.100     | 0.460 | 0.370 | 1.758          | 0.415        | 3.133          | 0.209        |
| 81  | 0.670     | 0.480 | 23.0 | 0.170 | 0.700     | 0.000 | 0.125 | <b>7.731</b>   | <b>0.021</b> | 4.800          | 0.091        |
| 82  | 0.240     | 0.290 | 18.0 | 0.640 | 0.000     | 0.000 | 0.050 | 4.364          | 0.113        | 4.800          | 0.091        |
| 83  | 1.000     | 1.000 | —    | —     | 0.880     | 0.750 | 0.500 | 4.476          | 0.107        | <b>7.200</b>   | <b>0.027</b> |
| 84  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.400          | 0.497        | n.a.           | n.a.         |
| 85  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 86  | 0.465     | 0.820 | 5.0  | 0.083 | 0.300     | 0.420 | 0.315 | 1.805          | 0.406        | 0.533          | 0.766        |
| 87  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 88  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 89  | 0.905     | 1.000 | 13.5 | 0.852 | 1.000     | 0.630 | 0.825 | <b>7.082</b>   | <b>0.029</b> | 4.800          | 0.091        |
| 90  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 91  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 92  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 93  | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 94  | 0.615     | 0.710 | 16.0 | 0.927 | 0.670     | 0.500 | 0.725 | 4.751          | 0.093        | 3.133          | 0.209        |
| 95  | 0.200     | 0.310 | 16.5 | 0.855 | 0.000     | 0.000 | 0.025 | 2.783          | 0.249        | 3.111          | 0.211        |
| 96  | 0.020     | 0.000 | 22.5 | 0.104 | 0.000     | 0.000 | 0.000 | 3.000          | 0.223        | 3.273          | 0.195        |
| 97  | 1.000     | 0.960 | 18.0 | 0.619 | 0.500     | 0.500 | 0.500 | 1.431          | 0.489        | n.a.           | n.a.         |
| 98  | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 99  | 0.000     | 0.000 | 17.5 | 0.465 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 100 | 1.000     | 1.000 | 10.0 | 0.223 | 0.500     | 0.500 | 0.500 | 3.000          | 0.223        | 3.273          | 0.195        |
| 101 | 1.000     | 1.000 | 16.0 | 0.916 | 1.000     | 0.800 | 1.000 | 5.137          | 0.077        | 3.133          | 0.209        |
| 102 | 0.030     | 0.000 | 21.0 | 0.245 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 103 | 1.000     | 1.000 | 10.0 | 0.223 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 104 | 0.630     | 0.550 | 19.0 | 0.523 | 0.750     | 0.900 | 0.735 | 2.939          | 0.230        | 3.133          | 0.209        |
| 105 | 1.000     | 1.000 | —    | —     | 1.000     | 1.000 | 1.000 | 1.829          | 0.401        | n.a.           | n.a.         |
| 106 | 0.310     | 0.140 | 23.0 | 0.170 | 0.500     | 0.500 | 0.535 | 4.716          | 0.095        | 4.800          | 0.091        |
| 107 | 0.000     | 0.000 | 17.5 | 0.465 | 0.500     | 0.500 | 0.500 | 3.000          | 0.223        | n.a.           | n.a.         |
| 108 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | 1.804          | 0.406        | n.a.           | n.a.         |
| 109 | 0.670     | 0.560 | 14.0 | 0.834 | 0.800     | 0.630 | 0.810 | 3.048          | 0.218        | 3.133          | 0.209        |
| 110 | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 111 | 0.810     | 0.880 | 9.5  | 0.360 | 1.000     | 1.000 | 0.920 | <b>7.748</b>   | <b>0.021</b> | n.a.           | n.a.         |
| 112 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 113 | 0.275     | 0.500 | 8.0  | 0.235 | 0.500     | 1.000 | 0.845 | <b>9.469</b>   | <b>0.009</b> | <b>9.000</b>   | <b>0.011</b> |
| 114 | 0.000     | 0.000 | 17.5 | 0.465 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 115 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 116 | 0.500     | 0.850 | 3.0  | 0.060 | 0.500     | 0.450 | 0.410 | 0.586          | 0.746        | 1.253          | 0.535        |
| 117 | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 118 | 0.000     | 0.000 | —    | —     | 0.250     | 0.500 | 0.500 | <b>10.730</b>  | <b>0.005</b> | n.a.           | n.a.         |
| 119 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 120 | 0.570     | 0.820 | 7.0  | 0.170 | 0.400     | 0.500 | 0.395 | 2.270          | 0.321        | 3.600          | 0.165        |
| 121 | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 122 | 1.000     | 1.000 | 18.0 | 0.361 | 0.580     | 0.500 | 0.500 | 5.776          | 0.056        | <b>7.200</b>   | <b>0.027</b> |
| 123 | 0.895     | 1.000 | 7.5  | 0.103 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 124 | 0.000     | 0.000 | 20.0 | 0.223 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 125 | 0.000     | 0.000 | 20.0 | 0.223 | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 126 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 127 | 1.000     | 1.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |
| 128 | 0.000     | 0.000 | —    | —     | 0.500     | 0.500 | 0.500 | —              | —            | —              | —            |

| SNP        | RR        |       |      |       | LR        |       |       |                |       |                |       |
|------------|-----------|-------|------|-------|-----------|-------|-------|----------------|-------|----------------|-------|
|            | $\bar{x}$ |       | MWW  |       | $\bar{x}$ |       |       | Kruskal-Wallis |       | Mood's Median  |       |
|            | R         | R-E   | W    | p     | L-E       | E     | R-E   | H              | p     | X <sup>2</sup> | p     |
| <b>129</b> | 1.000     | 1.000 | 12.5 | 0.465 | 0.500     | 0.500 | 0.500 | 1.335          | 0.513 | n.a.           | n.a.  |
| <b>130</b> | 1.000     | 1.000 | 21.0 | 0.136 | 0.500     | 0.500 | 0.500 | 2.000          | 0.368 | n.a.           | n.a.  |
| <b>131</b> | 0.000     | 0.000 | 9.0  | 0.136 | 0.500     | 0.500 | 0.500 | 2.000          | 0.368 | 2.182          | 0.336 |

**Table S9.** Single nucleotide polymorphisms (SNPs) of gametogenic genes associated with the categorical variable population system (POPSYS) based on logistic regression. SNPs causing amino acid substitutions are shown in **bold**. To test for potential geographic effects on SNP frequency (f), the quantitative factors latitude (LAT) and longitude (LON) were added to the model. The smallest frequency proportion allowed was 1E-10. The Kruskal-Wallis (K-W) tests revealed significant system-specific differences for all SNPs except SNP50. Logistic regressions identified a significant effect of POPSYS on f for all SNPs except SNP24. The logistic models were based on type III sum of squares; forward selection models are indicated with \*. R<sup>2</sup> represents the percentage of variability in SNP frequencies explained by the regression model; R<sup>2</sup><sub>adj</sub> allows for comparisons of models with different numbers of coefficients. Cases where the logistic function did not fit the observed data are noted by p-values < 0.05 in the X<sup>2</sup> statistic. d.f.: degrees of freedom. L-E: *lessonae-esculentus* populations, R-E: *ridibundus-esculentus* populations, E: all-hybrid (*esculentus*) populations.

| Gene            | Exon | SNP | Logistic regression (Weighted Least Squares) |        |                    |                                   |                    |                         |       |      |        | Median (f) |       |       | K-W   |       |
|-----------------|------|-----|----------------------------------------------|--------|--------------------|-----------------------------------|--------------------|-------------------------|-------|------|--------|------------|-------|-------|-------|-------|
|                 |      |     | Model (ANOVA)                                |        |                    |                                   |                    | Type III Sum of Squares |       |      |        |            |       |       |       |       |
|                 |      |     | F                                            | p      | R <sup>2</sup> [%] | R <sup>2</sup> <sub>adj</sub> [%] | p(X <sup>2</sup> ) | Variable                | F     | d.f. | p      | L-E        | E     | R-E   | H     | p     |
| <i>fbxo5</i>    | 1    | 23  | 13.74                                        | 0.002  | 88.7               | 82.2                              | 0.956              | POPSYS                  | 14.58 | 2    | 0.003  | 0.750      | 0.500 | 0.500 | 9.13  | 0.010 |
| <i>hat1</i>     | 9    | 24  | 3.58                                         | 0.068  | 67.2               | 48.4                              | 0.764              | POPSYS                  | 4.46  | 2    | 0.056  | 0.500      | 0.500 | 0.550 | 7.16  | 0.028 |
| <i>henmt1</i>   | 6    | 28  | 5.25                                         | 0.028  | 75.0               | 60.7                              | <0.001             | LAT                     | 7.33  | 1    | 0.030  | 0.200      | 0.000 | 0.030 | 8.82  | 0.012 |
|                 |      |     |                                              |        |                    |                                   |                    | POPSYS                  | 9.93  | 2    | 0.009  |            |       |       |       |       |
| <i>hormad1</i>  | 7    | 31  | 33.62                                        | <0.001 | 95.0               | 92.2                              | 0.992              | LAT                     | 9.09  | 1    | 0.020  | 0.880      | 0.500 | 0.500 | 8.44  | 0.015 |
|                 |      |     |                                              |        |                    |                                   |                    | LON                     | 15.17 | 1    | 0.006  |            |       |       |       |       |
|                 |      |     |                                              |        |                    |                                   |                    | POPSYS                  | 45.02 | 2    | <0.001 |            |       |       |       |       |
| <i>kif22</i>    | 2    | 47  | 44.46*                                       | <0.001 | 90.8               | 88.8                              | 0.098              | POPSYS                  | 44.46 | 2    | <0.001 | 0.500      | 0.000 | 0.020 | 7.48  | 0.024 |
|                 | 5    | 48  | 6.62                                         | 0.016  | 79.1               | 67.2                              | 0.114              | POPSYS                  | 10.84 | 2    | 0.007  | 0.500      | 0.270 | 0.145 | 6.48  | 0.039 |
|                 |      | 49  | 6.71                                         | 0.015  | 79.3               | 67.5                              | 0.011              | POPSYS                  | 10.20 | 2    | 0.008  | 0.500      | 0.250 | 0.160 | 6.41  | 0.040 |
|                 | 6    | 50  | 13.97*                                       | 0.002  | 75.6               | 70.2                              | 0.054              | POPSYS                  | 13.97 | 2    | 0.002  | 1.000      | 0.750 | 0.905 | 1.86  | 0.394 |
|                 | 6    | 51  | 6.71                                         | 0.015  | 79.3               | 67.5                              | 0.221              | POPSYS                  | 10.20 | 2    | 0.008  | 0.500      | 0.750 | 0.840 | 6.41  | 0.040 |
| <i>mns1</i>     | 7    | 74  | 3.38                                         | 0.077  | 65.9               | 46.4                              | 0.831              | POPSYS                  | 5.52  | 2    | 0.036  | 0.830      | 0.500 | 0.380 | 7.38  | 0.025 |
| <i>nusap1</i>   | 8    | 81  | 48.93*                                       | <0.001 | 94.8               | 92.9                              | 0.344              | LAT                     | 11.15 | 1    | 0.010  | 0.700      | 0.000 | 0.125 | 7.73  | 0.021 |
|                 |      |     |                                              |        |                    |                                   |                    | POPSYS                  | 20.37 | 2    | <0.001 |            |       |       |       |       |
| <i>rad50</i>    | 5    | 111 | 44.64                                        | <0.001 | 96.2               | 94.1                              | 0.979              | POPSYS                  | 10.57 | 2    | 0.008  | 1.000      | 1.000 | 0.920 | 7.75  | 0.021 |
| <i>rad51ap1</i> | 5    | 113 | 6.82*                                        | 0.014  | 71.9               | 61.4                              | 0.945              | LON                     | 6.10  | 1    | 0.039  | 0.500      | 1.000 | 0.845 | 9.47  | 0.009 |
|                 |      |     |                                              |        |                    |                                   |                    | POPSYS                  | 6.75  | 2    | 0.019  |            |       |       |       |       |
| <i>sfr1</i>     | 2    | 118 | 11.80                                        | 0.003  | 87.1               | 79.7                              | >0.999             | POPSYS                  | 12.81 | 2    | 0.005  | 0.250      | 0.500 | 0.500 | 10.73 | 0.005 |

**Table S10.** Results of logistic regression using the frequencies of single nucleotide polymorphisms (SNPs) of diploid *P. esculentus* (genotype LR) as dependent variables. The predictor variables were population system (POPSYS), longitude (LON), and latitude (LAT). The table contains only those SNPs that were significantly affected by one or more predictor variables, as indicated by the type III sum of squares statistics. Model (M) variants: a – includes all independent variables, f – forward selection, b – backward selection.  $R^2$  represents the percentage of variability in SNP frequencies explained by the regression model;  $R^2_{adj}$  allows for comparisons of models with different numbers of coefficients. In some cases, the logistic function did not fit the observed data ( $X^2 < 0.05$ ), or the goodness-of-fit test could not be performed due to insufficient data (n.a.). System-specific median differences in SNP frequencies were evaluated using Kruskal-Wallis (K-W) tests; significant differences are highlighted in bold. L-E: *lessonae-esculentus* populations, E: all-hybrid (*esculentus*) populations, R-E: *ridibundus-esculentus* populations.

| Gene    | Exon  | SNP | Logistic regression (Weighted Least Squares) |        |        |                |                               |                |                         |        |       |        | Median |       |       | K-W   |       |       |
|---------|-------|-----|----------------------------------------------|--------|--------|----------------|-------------------------------|----------------|-------------------------|--------|-------|--------|--------|-------|-------|-------|-------|-------|
|         |       |     | Model (Analysis of Variance)                 |        |        |                |                               |                | Type III Sum of Squares |        |       |        | POPSYS |       |       |       |       |       |
|         |       |     | M                                            | F      | p      | R <sup>2</sup> | R <sup>2</sup> <sub>adj</sub> | X <sup>2</sup> | Variable                | F      | d.f.  | p      | L-E    | E     | R-E   | H     | p     |       |
| brd2    | 2     | 3   | f,b                                          | 7.49   | 0.012  | 62.5           | 54.1                          | n.a.           | POPSYS                  | 7.49   | 1     | 0.012  | 0.500  | 0.500 | 0.550 | 1.783 | 0.410 |       |
|         | 4     | 4   | f,b                                          | 33.32  | <0.001 | 93.5           | 90.6                          | 0.129          | LON                     | 12.61  | 1     | 0.009  | 0.630  | 0.625 | 0.865 | 1.38  | 0.500 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 40.39  | 2     | <0.001 |        |       |       |       |       |       |
| camk2g  | 5     | 5   | f,b                                          | 5.50   | 0.028  | 55.0           | 45.0                          | n.a.           | POPSYS                  | 5.50   | 2     | 0.028  | 0.630  | 0.890 | 0.875 | 0.89  | 0.640 |       |
|         | 19    | 9   | b                                            | 11.18  | 0.003  | 80.7           | 73.5                          | 0.373          | LAT                     | 25.19  | 1     | 0.001  | 0.900  | 1.000 | 0.855 | 1.60  | 0.449 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 16.75  | 2     | 0.001  |        |       |       |       |       |       |
| fbxo5   | 1     | 23  | a                                            | 13.74  | 0.002  | 88.7           | 82.2                          | 0.956          | POPSYS                  | 14.58  | 2     | 0.003  | 0.750  | 0.500 | 0.500 | 9.13  | 0.010 |       |
| hat1    | 9     | 24  | f,b                                          | 5.24   | 0.031  | 53.8           | 43.6                          | n.a.           | POPSYS                  | 5.24   | 2     | 0.031  | 0.500  | 0.500 | 0.550 | 7.16  | 0.028 |       |
| henmt   | 6     | 28  | a                                            | 4.58   | 0.039  | 72.4           | 56.6                          | 0.0            | LAT                     | 5.82   | 1     | 0.047  | 0.200  | 0.000 | 0.030 | 8.82  | 0.012 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 8.49   | 2     | 0.013  |        |       |       |       |       |       |
| hormad1 | 7     | 31  | a,b                                          | 33.62  | <0.001 | 95.0           | 92.2                          | 0.992          | LAT                     | 9.09   | 1     | 0.020  |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 15.17  | 1     | 0.006  | 0.880  | 0.500 | 0.500 | 8.44  | 0.015 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 45.02  | 2     | <0.001 |        |       |       |       |       |       |
| igf1r   | 6     | 34  | f,b                                          | 41.89  | <0.001 | 94.0           | 91.8                          | 0.840          | LON                     | 103.67 | 1     | <0.001 | 1.000  | 1.000 | 0.900 | 5.19  | 0.075 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 20.77  | 2     | <0.001 |        |       |       |       |       |       |
| itpr1   | 5     | 37  | a,f,b                                        | 66.62  | <0.001 | 97.4           | 96.0                          | <0.001         | LAT                     | 84.63  | 1     | <0.001 | 1.000  | 1.000 | 0.940 | 1.83  | 0.401 |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 250.01 | 1     | <0.001 |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 16.53  | 2     | 0.002  |        |       |       |       |       |       |
|         | 21    | 42  | a,b                                          | 123.45 | <0.001 | 98.6           | 97.8                          | 0.951          | LAT                     | 8.14   | 1     | 0.025  |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 10.90  | 1     | 0.013  | 0.000  | 0.000 | 0.025 | 2.61  | 0.271 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 17.90  | 2     | 0.002  |        |       |       |       |       |       |
|         |       | 44  | f,b                                          | 9.14   | 0.007  | 67.0           | 59.7                          | 0.953          | LAT                     | 17.31  | 1     | 0.002  | 0.500  | 0.500 | 0.500 | 3.00  | 0.223 |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 8.48   | 1     | 0.017  |        |       |       |       |       |       |
|         | 29    | 45  | f,b                                          | 15.50  | 0.001  | 77.5           | 72.5                          | 0.797          | LAT                     | 29.82  | 1     | <0.001 | 0.500  | 0.500 | 0.500 | 3.00  | 0.223 |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 15.50  | 1     | 0.003  |        |       |       |       |       |       |
|         | kif22 | 2   | 47                                           | f,b    | 42.31  | <0.001         | 90.4                          | 88.2           | 0.099                   | POPSYS | 42.31 | 2      | <0.001 | 0.500 | 0.000 | 0.020 | 7.48  | 0.024 |
|         |       | 5   | 48                                           | a      | 6.55   | 0.016          | 78.9                          | 66.9           | 0.114                   | POPSYS | 10.73 | 2      | 0.007  | 0.500 | 0.270 | 0.145 | 6.48  | 0.039 |
|         |       | 49  | a                                            | 6.64   | 0.016  | 79.1           | 67.2                          | 0.011          | POPSYS                  | 10.10  | 2     | 0.009  | 0.500  | 0.250 | 0.160 | 6.41  | 0.040 |       |
| 6       |       | 50  | a,b                                          | 221.83 | <0.001 | 99.2           | 98.8                          | 0.016          | LAT                     | 198.84 | 1     | <0.001 |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 214.11 | 1     | <0.001 |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 198.45 | 2     | <0.001 | 1.000  | 0.750 | 0.905 | 1.86  | 0.394 |       |
|         |       |     | f                                            | 13.60  | 0.002  | 75.1           | 69.6                          | 0.054          | POPSYS                  | 13.60  | 2     | 0.002  |        |       |       |       |       |       |
|         | 51    | a   | 6.64                                         | 0.016  | 79.2   | 67.2           | 0.222                         | POPSYS         | 10.10                   | 2      | 0.009 | 0.500  | 0.750  | 0.840 | 6.41  | 0.040 |       |       |
| kmt2a   | 7     | 52  | a                                            | 1.50   | 0.298  | 46.2           | 15.5                          | 0.547          | —                       | —      | —     | 0.750  | 0.500  | 0.500 | 7.49  | 0.024 |       |       |
| m1ap    | 1     | 57  | b                                            | 6.68   | 0.014  | 71.5           | 60.8                          | 0.990          | LAT                     | 10.38  | 1     | 0.012  | 0.500  | 0.500 | 0.525 | 4.72  | 0.095 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 5.58   | 2     | 0.030  |        |       |       |       |       |       |
| map2k1  | 3     | 59  | f,b                                          | 103.43 | <0.001 | 95.8           | 94.9                          | 1.000          | LAT                     | 131.47 | 1     | <0.001 | 1.000  | 1.000 | 1.000 | 3.00  | 0.223 |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 10.28  | 1     | 0.011  |        |       |       |       |       |       |
| mns1    | 7     | 74  | a                                            | 3.38   | 0.077  | 65.9           | 46.4                          | 0.830          | POPSYS                  | 5.52   | 2     | 0.036  | 0.830  | 0.500 | 0.380 | 7.38  | 0.025 |       |
|         |       |     | f,b                                          | 8.57   | 0.008  | 65.6           | 57.9                          | n.a.           | POPSYS                  | 8.57   | 2     | 0.008  |        |       |       |       |       |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 8.88   | 1     | 0.014  | 0.100  | 0.460 | 0.370 | 1.76  | 0.415 |       |
| nibrin  | 10    | 80  | f,b                                          | 8.88   | 0.014  | 47.0           | 41.7                          | 0.592          | LON                     | 8.88   | 1     | 0.014  | 0.100  | 0.460 | 0.370 | 1.76  | 0.415 |       |
| nusap1  | 8     | 81  | f,b                                          | 48.93  | <0.001 | 94.8           | 92.9                          | 0.344          | LAT                     | 11.15  | 1     | 0.010  | 0.700  | 0.000 | 0.125 | 7.73  | 0.021 |       |
|         |       |     |                                              |        |        |                |                               |                | POPSYS                  | 20.37  | 2     | <0.001 |        |       |       |       |       |       |
|         |       | 82  | f,b                                          | 44.36  | <0.001 | 94.3           | 92.2                          | n.a            | LAT                     | 115.34 | 1     | <0.001 | 0.000  | 0.000 | 0.050 | 4.36  | 0.113 |       |
|         |       |     |                                              |        |        |                |                               | POPSYS         | 11.67                   | 2      | 0.004 |        |        |       |       |       |       |       |
| piwil2  | 12    | 89  | f,b                                          | 11.13  | 0.008  | 52.7           | 47.9                          | 0.042          | LON                     | 11.13  | 1     | 0.008  | 1.000  | 0.630 | 0.825 | 7.08  | 0.029 |       |
| pkmyt1  | 5     | 94  | f,b                                          | 6.85   | 0.026  | 40.7           | 34.7                          | n.a.           | LAT                     | 6.85   | 1     | 0.026  | 0.670  | 0.500 | 0.725 | 4.75  | 0.093 |       |
|         |       |     |                                              |        |        |                |                               |                | LAT                     | 46.38  | 1     | <0.001 |        |       |       |       |       |       |
|         |       | 95  | b                                            | 20.10  | <0.001 | 88.3           | 83.9                          | n.a.           | POPSYS                  | 26.10  | 2     | <0.001 | 0.000  | 0.000 | 0.025 | 2.78  | 0.249 |       |
| plk1    | 1     | 96  | f,b                                          | 108.72 | <0.001 | 96.0           | 95.1                          | 0.958          | LAT                     | 138.87 | 1     | <0.001 | 0.000  | 0.000 | 0.000 | 3.00  | 0.223 |       |
|         |       |     |                                              |        |        |                |                               |                | LON                     | 11.12  | 1     | 0.009  |        |       |       |       |       |       |

| Gene     | Exon  | SNP | Logistic regression (Weighted Least Squares) |       |        |                |                               |                |                         |        |        |        | Median |       |       | K-W   |       |        |
|----------|-------|-----|----------------------------------------------|-------|--------|----------------|-------------------------------|----------------|-------------------------|--------|--------|--------|--------|-------|-------|-------|-------|--------|
|          |       |     | Model (Analysis of Variance)                 |       |        |                |                               |                | Type III Sum of Squares |        |        |        | POPSYS |       |       |       |       |        |
|          |       |     | M                                            | F     | p      | R <sup>2</sup> | R <sup>2</sup> <sub>adj</sub> | X <sup>2</sup> | Variable                | F      | d.f.   | p      | L-E    | E     | R-E   | H     | p     |        |
| ppp1ca   | 3     | 100 | f,b                                          | 10.65 | 0.004  | 70.3           | 63.7                          | 0.760          | LAT                     | 20.21  | 1      | 0.002  | 0.500  | 0.500 | 0.500 | 3.00  | 0.223 |        |
|          |       |     | 101                                          | a,b   | 50.13  | <0.001         | 96.6                          | 94.7           | n.a.                    | LAT    | 65.94  | 1      |        |       |       |       |       | <0.001 |
|          |       |     |                                              |       |        |                |                               |                |                         | LON    | 5.69   | 1      |        |       |       |       |       | 0.048  |
|          | 104   | b   | 4.39                                         | 0.042 | 62.2   | 48.1           | n.a.                          | POPSYS         | 29.88                   | 2      | <0.001 | 1.000  | 0.800  | 1.000 | 5.14  | 0.077 |       |        |
|          |       |     |                                              |       |        |                |                               | LON            | 18.12                   | 1      | 0.002  |        |        |       |       |       |       |        |
|          |       |     |                                              |       |        |                |                               | LON            | 12.56                   | 1      | 0.008  |        |        |       |       |       |       |        |
| ppp2r1a  | 4     | 105 | b                                            | 10.27 | 0.004  | 79.4           | 71.7                          | n.a.           | POPSYS                  | 5.07   | 2      | 0.038  | 0.750  | 0.900 | 0.735 | 2.94  | 0.230 |        |
|          |       |     |                                              |       |        |                |                               |                | LON                     | 29.98  | 1      | <0.001 |        |       |       |       |       |        |
|          |       |     |                                              |       |        |                |                               |                | POPSYS                  | 15.02  | 2      | 0.002  |        |       |       |       |       |        |
|          | 106   | b   | 6.94                                         | 0.013 | 72.2   | 61.8           | n.a.                          | LAT            | 9.23                    | 1      | 0.016  | 0.500  | 0.535  | 0.500 | 4.72  | 0.095 |       |        |
|          |       |     |                                              |       |        |                |                               | POPSYS         | 8.93                    | 2      | 0.009  |        |        |       |       |       |       |        |
|          |       |     |                                              |       |        |                |                               | LON            | 28.08                   | 1      | <0.001 |        |        |       |       |       |       |        |
| ppp3ca   | 2     | 109 | b                                            | 28.08 | <0.001 | 73.7           | 71.1                          | 0.363          | POPSYS                  | 10.57  | 2      | 0.008  | 0.800  | 0.630 | 0.810 | 3.04  | 0.218 |        |
|          | rad50 | 5   | 111                                          | a     | 44.64  | <0.001         | 96.2                          | 94.1           | 0.979                   | LON    | 28.08  | 1      | <0.001 | 1.000 | 1.000 | 0.920 | 7.75  | 0.021  |
| LON      |       |     |                                              |       |        |                |                               |                |                         | 109.96 | 1      | <0.001 |        |       |       |       |       |        |
| POPSYS   |       |     |                                              |       |        |                |                               |                |                         | 6.51   | 2      | 0.021  |        |       |       |       |       |        |
| rad51ap1 | 5     | 113 | b                                            | 63.32 | <0.001 | 96.0           | 94.4                          | 0.987          | LAT                     | 168.56 | 1      | <0.001 | 0.500  | 1.000 | 0.845 | 9.47  | 0.009 |        |
|          |       |     |                                              |       |        |                |                               |                | POPSYS                  | 65.77  | 2      | <0.001 |        |       |       |       |       |        |
|          |       |     |                                              |       |        |                |                               |                | LON                     | 6.97   | 1      | 0.033  |        |       |       |       |       |        |
|          | sfr1  | 2   | 118                                          | a     | 11.80  | 0.003          | 87.1                          | 79.7           | 1.000                   | LON    | 6.10   | 1      | 0.039  | 0.500 | 0.500 | 0.500 | 10.73 | 0.005  |
|          |       |     |                                              |       |        |                |                               |                |                         | POPSYS | 6.75   | 2      | 0.019  |       |       |       |       |        |
|          |       |     |                                              |       |        |                |                               |                |                         | LON    | 6.95   | 1      | 0.025  |       |       |       |       |        |
| sgol1    | 2     | 122 | f,b                                          | 5.17  | 0.032  | 53.5           | 43.1                          | n.a.           | POPSYS                  | 5.17   | 2      | 0.032  | 0.400  | 0.500 | 0.395 | 2.27  | 0.321 |        |
|          |       |     |                                              |       |        |                |                               |                | LAT                     | 6.95   | 1      | 0.025  | 0.580  | 0.500 | 0.500 | 5.78  | 0.056 |        |



**Table S11.** Results of logistic regression using the frequencies of single nucleotide polymorphisms (SNPs) of *P. ridibundus* (genotype RR) as dependent variables. The predictor variables were population system (POPSYS), longitude (LON), and latitude (LAT). The table contains only those SNPs that were significantly affected by one or more predictor variables, as indicated by the type III sum of squares statistics. Model (M) variants: a – includes all independent variables, f – forward selection, b – backward selection.  $R^2$  represents the percentage of variability in SNP frequencies explained by the regression model;  $R^2_{adj}$  allows for comparisons of models with different numbers of coefficients. In some cases, the logistic function did not fit the observed data ( $X^2 < 0.05$ ), or the goodness-of-fit test could not be performed due to insufficient data (n.a.). System-specific median differences in SNP frequencies were evaluated using Mann-Whitney-Wilcoxon (MWW) tests; significant differences are highlighted in bold. R: all-*ridibundus* populations, R-E: *ridibundus-esculentus* populations.

| Gene    | Exon | SNP | Logistic regression (Weighted Least Squares) |       |        |                |                               |                     |                         |        |      |        | Median |      | MWW  |       |
|---------|------|-----|----------------------------------------------|-------|--------|----------------|-------------------------------|---------------------|-------------------------|--------|------|--------|--------|------|------|-------|
|         |      |     | Model (Analysis of Variance)                 |       |        |                |                               |                     | Type III Sum of Squares |        |      |        | POPSYS |      |      |       |
|         |      |     | M                                            | F     | p      | R <sup>2</sup> | R <sup>2</sup> <sub>adj</sub> | p (X <sup>2</sup> ) | Variable                | F      | d.f. | p      | R      | R-E  | W    | p     |
| adcy9   | 6    | 1   | f                                            | 20.57 | 0.001  | 69.6           | 66.2                          | 0.406               | LON                     | 20.57  | 1    | 0.001  | 0.0    | 0.0  | 9.0  | 0.136 |
|         |      |     | b                                            | 14.66 | 0.002  | 78.6           | 73.2                          | 0.530               | LAT                     | 12.71  | 1    | 0.007  |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 13.01  | 1    | 0.007  |        |      |      |       |
|         | 2    |     | f                                            | 20.57 | 0.001  | 69.6           | 66.2                          | 0.406               | LON                     | 20.57  | 1    | 0.001  | 0.0    | 0.0  | 9.0  | 0.136 |
|         |      |     | b                                            | 14.66 | 0.002  | 78.6           | 73.2                          | 0.530               | LAT                     | 12.71  | 1    | 0.007  |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 13.01  | 1    | 0.007  |        |      |      |       |
| brd2    | 2    | 3   | f                                            | 9.21  | 0.016  | 53.5           | 47.7                          | 0.598               | LON                     | 9.21   | 1    | 0.016  | 0.13   | 0.17 | 5.0  | 0.141 |
|         | 5    | 5   | f                                            | 9.18  | 0.014  | 50.5           | 45.0                          | 0.142               | LON                     | 9.18   | 1    | 0.014  | 0.92   | 0.80 | 25.0 | 0.082 |
| camk2g  | 19   | 9   | a                                            | 72.3  | <0.001 | 97.3           | 96.0                          | <0.001              | LAT                     | 75.55  | 1    | <0.001 | 0.83   | 0.50 | 13.0 | 1.0   |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 51.08  | 1    | <0.001 |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 150.19 | 1    | <0.001 |        |      |      |       |
| cdc20   | 1    | 12  | f                                            | 97.11 | <0.001 | 91.5           | 90.6                          | 0.949               | LAT                     | 97.11  | 1    | <0.001 | 1.0    | 1.0  | 12.5 | 0.465 |
| fbxo43  | 5    | 22  | a                                            | 92.40 | <0.001 | 97.5           | 96.5                          | 0.938               | LAT                     | 18.83  | 1    | 0.003  | 1.0    | 1.0  | 12.5 | 0.465 |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 57.36  | 1    | <0.001 |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 9.97   | 1    | 0.016  |        |      |      |       |
| hat1    | 9    | 24  | f                                            | 18.14 | 0.001  | 81.9           | 77.4                          | 0.987               | LON                     | 13.14  | 1    | 0.007  | 0.0    | 0.0  | 17.5 | 0.465 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 13.32  | 1    | 0.006  |        |      |      |       |
| henmt1  | 3    | 25  | f                                            | 13.18 | 0.006  | 59.4           | 54.9                          | n.a.                | POPSYS                  | 13.18  | 1    | 0.006  | 0.0    | 0.0  | 12.0 | 0.361 |
|         | 4    | 27  | b                                            | 4.10  | 0.059  | 50.6           | 38.3                          | 0.698               | LAT                     | 5.33   | 1    | <0.050 | 0.55   | 0.44 | 19.0 | 0.523 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 7.11   | 1    | 0.028  |        |      |      |       |
| hormad1 | 7    | 30  | a                                            | 4.69  | 0.042  | 66.8           | 52.5                          | 0.351               | POPSYS                  | 6.18   | 1    | 0.042  | 0.0    | 0.0  | 9.0  | 0.134 |
|         |      | 31  | a                                            | 4.69  | 0.042  | 66.8           | 52.5                          | 0.432               | POPSYS                  | 6.18   | 1    | 0.042  | 1.0    | 1.0  | 21.0 | 0.134 |
|         |      | 32  | a                                            | 4.69  | 0.042  | 66.8           | 52.5                          | 0.351               | POPSYS                  | 6.18   | 1    | 0.042  | 0.0    | 0.0  | 9.0  | 0.134 |
| igf1r   | 6    | 34  | b                                            | 10.18 | 0.006  | 71.8           | 64.7                          | 0.003               | LAT                     | 10.47  | 1    | 0.012  | 0.94   | 0.83 | 20.0 | 0.409 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 19.84  | 1    | 0.002  |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 17.12  | 1    | 0.003  |        |      |      |       |
|         | 21   | 42  | f                                            | 9.87  | 0.007  | 71.2           | 63.9                          | 0.008               | LON                     | 17.12  | 1    | 0.003  | 0.03   | 0.07 | 16.0 | 0.925 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 5.98   | 1    | 0.040  |        |      |      |       |
|         |      |     | a                                            | 5.98  | 0.024  | 71.9           | 59.9                          | 0.515               | POPSYS                  | 9.96   | 1    | 0.016  |        |      |      |       |
| m1ap    | 1    | 57  | a                                            | 60.36 | <0.001 | 96.3           | 94.7                          | <0.001              | LAT                     | 12.39  | 1    | 0.008  | 0.14   | 0.21 | 8.5  | 0.271 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 14.45  | 1    | 0.005  |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 29.40  | 1    | 0.001  |        |      |      |       |
| map3k7  | 17   | 61  | f                                            | 18.25 | 0.001  | 82.0           | 77.5                          | 0.992               | POPSYS                  | 12.29  | 1    | 0.010  | 0.0    | 0.0  | 17.5 | 0.465 |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 13.25  | 1    | 0.007  |        |      |      |       |
| mr11a   | 14   | 77  | f,b                                          | 90.44 | <0.001 | 90.9           | 89.9                          | 0.972               | LAT                     | 90.44  | 1    | <0.001 | 1.0    | 1.0  | 12.5 | 0.465 |
| msh2    | 3    | 78  | a,b                                          | 94.07 | <0.001 | 97.6           | 96.5                          | 0.998               | LAT                     | 19.08  | 1    | 0.003  | 0.95   | 1.0  | 12.5 | 0.465 |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 58.36  | 1    | <0.001 |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 9.98   | 1    | 0.016  |        |      |      |       |
| nusap1  | 8    | 81  | f                                            | 90.36 | <0.001 | 90.0           | 89.9                          | 0.756               | LON                     | 90.36  | 1    | <0.001 | 0.67   | 0.48 | 23.0 | 0.170 |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 9.05   | 1    | 0.015  |        |      |      |       |
|         |      |     |                                              |       |        |                |                               |                     | LON                     | 11.12  | 1    | 0.012  |        |      |      |       |
|         | 8    | 82  | a                                            | 14.05 | 0.002  | 85.8           | 79.6                          | <0.001              | LAT                     | 23.79  | 1    | 0.002  | 0.24   | 0.29 | 18.0 | 0.640 |
|         |      |     |                                              |       |        |                |                               |                     | POPSYS                  | 19.72  | 1    | 0.003  |        |      |      |       |
|         |      |     | f                                            | 7.54  | 0.023  | 45.6           | 39.5                          | n.a.                | LON                     | 7.54   | 1    | 0.023  |        |      |      |       |
| pelp1   | 4    | 86  | f                                            | 8.34  | 0.018  | 48.1           | 42.3                          | 0.073               | LAT                     | 8.34   | 1    | 0.018  | 0.44   | 0.82 | 5.0  | 0.083 |
|         |      |     | b                                            | 7.60  | 0.022  | 45.8           | 39.8                          | 0.154               | LON                     | 7.60   | 1    | 0.022  |        |      |      |       |
| piwil2  | 12   | 89  | a                                            | 8.16  | 0.011  | 77.8           | 68.2                          | <0.001              | POPSYS                  | 8.01   | 1    | 0.025  | 0.90   | 1.00 | 13.5 | 0.952 |
|         |      |     | f,b                                          | 31.15 | <0.001 | 77.6           | 75.1                          | n.a.                | POPSYS                  | 31.15  | 1    | <0.001 |        |      |      |       |

| Gene    | Exon   | SNP    | Logistic regression (Weighted Least Squares) |        |        |                |                               |                     |                         | Median |        |        |       | MWW   |       |       |       |
|---------|--------|--------|----------------------------------------------|--------|--------|----------------|-------------------------------|---------------------|-------------------------|--------|--------|--------|-------|-------|-------|-------|-------|
|         |        |        | Model (Analysis of Variance)                 |        |        |                |                               |                     | Type III Sum of Squares |        |        | POPSYS |       |       |       |       |       |
|         |        |        | M                                            | F      | p      | R <sup>2</sup> | R <sup>2</sup> <sub>adj</sub> | p (X <sup>2</sup> ) | Variable                | F      | d.f.   | p      | R     | R-E   | W     | p     |       |
| plk1    | 1      | 96     | a                                            | 195.15 | <0.001 | 98.8           | 98.3                          | 0.473               | LAT                     | 73.06  | 1      | <0.001 | 0.02  | 0.0   | 22.5  | 0.104 |       |
|         |        |        | LON                                          | 404.42 | 1      | <0.001         |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        |        | POPSYS                                       | 12.66  | 1      | 0.009          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         | 97     | a      | 34.14                                        | <0.001 | 93.6   | 90.9           | <0.001                        | LAT                 | 43.09                   | 1      | <0.001 | 1.0    | 0.96  | 18.0  | 0.619 |       |       |
|         |        | LON    | 70.22                                        | 1      | <0.001 |                |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        | POPSYS | 22.29                                        | 1      | 0.002  |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| 5       | 99     | f,b    | 93.19                                        | <0.001 | 91.2   | 90.2           | 0.937                         | LAT                 | 93.19                   | 1      | <0.001 | 0.0    | 0.0   | 17.5  | 0.465 |       |       |
|         | 100    | b      | 103.26                                       | <0.001 | 96.3   | 95.3           | 0.950                         | LAT                 | 22.45                   | 1      | 0.002  | 0.97   | 1.0   | 10.0  | 0.223 |       |       |
| LON     | 29.95  | 1      | <0.001                                       |        |        |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| ppp1ca  | 3      | 101    | a,f,b                                        | 70.79  | <0.001 | 96.8           | 95.4                          | <0.001              | LAT                     | 33.99  | 1      | <0.001 | 0.97  | 0.98  | 16.0  | 0.916 |       |
|         |        |        | LON                                          | 44.11  | 1      | <0.001         |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        |        | POPSYS                                       | 13.45  | 1      | 0.008          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         | 102    | a,f,b  | 56.96                                        | <0.001 | 96.1   | 94.4           | <0.001                        | LAT                 | 24.77                   | 1      | 0.002  | 0.03   | 0.0   | 21.0  | 0.245 |       |       |
|         |        | LON    | 20.91                                        | 1      | 0.003  |                |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        | POPSYS | 54.38                                        | 1      | <0.001 |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| 103     | f,b    | 103.26 | <0.001                                       | 96.3   | 95.3   | 0.950          | LAT                           | 22.45               | 1                       | 0.002  | 1.0    | 1.0    | 10.0  | 0.223 |       |       |       |
|         | LON    | 29.95  | 1                                            | <0.001 |        |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| ppp2r1a | 4      | 106    | f,b                                          | 28.03  | <0.001 | 75.7           | 73.0                          | 0.086               | LAT                     | 28.03  | 1      | <0.001 | 0.31  | 0.14  | 23.0  | 0.170 |       |
|         |        | 107    | f,b                                          | 18.19  | 0.001  | 82.0           | 77.5                          | 0.984               | LON                     | 13.24  | 1      | 0.007  | 0.0   | 0.0   | 17.5  | 0.465 |       |
|         |        | POPSYS | 13.28                                        | 1      | 0.006  |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| ppp3ca  | 2      | 109    | a,b                                          | 4.14   | 0.066  | 67.4           | 51.1                          | 0.132               | LAT                     | 10.14  | 1      | 0.019  | 0.67  | 0.56  | 14.0  | 0.834 |       |
|         |        |        | LON                                          | 8.96   | 1      | 0.024          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        |        | POPSYS                                       | 12.12  | 1      | 0.013          |                               |                     |                         |        |        |        |       |       |       |       |       |
| rbbp8   | 6      | 114    | f,b                                          | 18.24  | 0.001  | 82.0           | 77.5                          | 0.991               | LON                     | 13.24  | 1      | 0.007  | 0.0   | 0.0   | 17.5  | 0.465 |       |
|         |        |        | POPSYS                                       | 13.35  | 1      | 0.006          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        |        | POPSYS                                       | 13.35  | 1      | 0.006          |                               |                     |                         |        |        |        |       |       |       |       |       |
| rps6k3  | 17     | 116    | f,b                                          | 11.46  | 0.010  | 58.9           | 53.8                          | n.a.                | POPSYS                  | 11.46  | 1      | 0.010  | 0.50  | 0.85  | 3.0   | 0.060 |       |
| sgol1   | 2      | 122    | f,b                                          | 13.07  | 0.006  | 59.2           | 54.7                          | n.a.                | POPSYS                  | 13.07  | 1      | 0.006  | 1.0   | 1.0   | 18.0  | 0.361 |       |
| smc1a   | 4      | 123    | a,b                                          | 13.11  | 0.003  | 84.9           | 78.4                          | <0.001              | LAT                     | 16.98  | 1      | 0.004  | 0.90  | 1.0   | 7.5   | 0.103 |       |
|         |        |        | LON                                          | 26.81  | 1      | 0.001          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        |        | POPSYS                                       | 11.51  | 1      | 0.012          |                               |                     |                         |        |        |        |       |       |       |       |       |
|         | 124    | f,b    | 68.41                                        | <0.001 | 94.5   | 93.1           | <0.001                        | LON                 | 127.50                  | 1      | <0.001 | 0.0    | 0.0   | 20.0  | 0.223 |       |       |
|         |        | POPSYS | 10.29                                        | 1      | 0.012  |                |                               |                     |                         |        |        |        |       |       |       |       |       |
|         |        | LON    | 127.50                                       | 1      | <0.001 |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| 125     | f,b    | 68.41  | <0.001                                       | 94.5   | 93.1   | <0.001         | LON                           | 10.29               | 1                       | 0.012  | 0.0    | 0.0    | 20.0  | 0.223 |       |       |       |
|         | POPSYS | 10.29  | 1                                            | 0.012  |        |                |                               |                     |                         |        |        |        |       |       |       |       |       |
| ywhaz   | 1      | 129    | f,b                                          | 18.98  | 0.001  | 82.6           | 78.2                          | 0.673               | LON                     | 13.69  | 1      | 0.006  | 1.0   | 1.0   | 12.5  | 0.465 |       |
| zfp36   | 2      | 130    | f,b                                          | 11.05  | 0.009  | 55.1           | 50.1                          | n.a.                | POPSYS                  | 11.05  | 1      | 0.009  | 1.0   | 1.0   | 21.0  | 0.136 |       |
|         |        |        | 131                                          | f,b    | 11.05  | 0.009          | 55.1                          | 50.1                | n.a.                    | POPSYS | 11.05  | 1      | 0.009 | 0.0   | 0.0   | 9.0   | 0.136 |

**Table S12.** Linkage disequilibrium ( $p$ -values) of single nucleotide polymorphisms (SNPs) selected for multi-locus genotype construction. Upper right matrix:  $p$ -values for the *P. lessonae* data set. Lower left matrix:  $p$ -values for the *P. ridibundus* data set. Blue and red shaded values indicate significantly linked loci for *P. lessonae* and *P. ridibundus*, respectively.

| Gene            | Gene | SNP | <i>fbxo5</i> | <i>hat1</i> | <i>henmt1</i> | <i>hormad1</i> | <i>kif22</i> | <i>kif22</i> | <i>kif22</i> | <i>kif22</i> | <i>kif22</i> | <i>mns1</i> | <i>nusap1</i> | <i>rad50</i> | <i>rad51ap1</i> | <i>sfr1</i> |
|-----------------|------|-----|--------------|-------------|---------------|----------------|--------------|--------------|--------------|--------------|--------------|-------------|---------------|--------------|-----------------|-------------|
|                 |      |     | 23           | 24          | 28            | 31             | 47           | 48           | 49           | 50           | 51           | 74          | 81            | 111          | 113             | 118         |
| <i>fbxo5</i>    | 23   |     |              | n.a.        | 0.1078        | 0.0622         | 0.0787       | 0.0035       | 0.0823       | 1.0000       | 0.0858       | 0.2096      | 0.0634        | 0.3528       | 0.0493          | 0.4500      |
| <i>hat1</i>     | 24   |     | 1.0000       |             | n.a.          | n.a.           | n.a.         | n.a.         | n.a.         | n.a.         | n.a.         | n.a.        | n.a.          | n.a.         | n.a.            | n.a.        |
| <i>henmt1</i>   | 28   |     | 1.0000       | 0.0003      |               | 1.0000         | 0.0000       | 0.0000       | 0.0000       | 0.0547       | 0.0000       | 0.0089      | 0.0000        | 0.0000       | 0.0000          | 0.2276      |
| <i>hormad1</i>  | 31   |     | 1.0000       | 0.0014      | 0.0000        |                | 0.0439       | 0.3963       | 0.1032       | 0.1713       | 0.1055       | 0.0060      | 0.0755        | 0.7035       | 0.1607          | 0.7360      |
| <i>kif22</i>    | 47   |     | 1.0000       | 0.0001      | 0.2227        | 0.0004         |              | 0.0000       | 0.0000       | 0.0637       | 0.0000       | 0.0000      | 0.0000        | 0.0023       | 0.0000          | 0.2782      |
| <i>kif22</i>    | 48   |     | 1.0000       | 0.0044      | 0.0000        | 0.0000         | 0.0000       |              | 0.0000       | 0.2704       | 0.0000       | 0.0000      | 0.0000        | 0.0000       | 0.0000          | 0.3840      |
| <i>kif22</i>    | 49   |     | 1.0000       | 0.0027      | 0.5582        | 0.0529         | 0.0000       | 0.0000       |              | 0.0878       | 0.0000       | 0.0001      | 0.0000        | 0.0141       | 0.0000          | 0.3241      |
| <i>kif22</i>    | 50   |     | 1.0000       | 0.0015      | 0.4779        | 0.2107         | 0.0019       | 0.0000       | 0.0000       |              | 0.2142       | 0.0989      | 0.2731        | 0.1342       | 0.3116          | 1.0000      |
| <i>kif22</i>    | 51   |     | 1.0000       | 0.0031      | 0.5687        | 0.0530         | 0.0000       | 0.0000       | 0.0000       | 0.0000       |              | n.a.        | n.a.          | n.a.         | n.a.            | n.a.        |
| <i>mns1</i>     | 74   |     | 1.0000       | 0.0026      | 0.0026        | 0.0050         | 0.0001       | 0.0001       | 0.0085       | 0.0026       | 0.0070       |             | 0.0000        | 0.0034       | 0.0000          | 0.9514      |
| <i>nusap1</i>   | 81   |     | 1.0000       | 0.0002      | 0.0002        | 0.0002         | 0.0000       | 0.0346       | 0.0749       | 0.0172       | 0.0742       | 0.0001      |               | 0.0033       | 0.0000          | 0.3764      |
| <i>rad50</i>    | 111  |     | 1.0000       | 0.0042      | 0.0000        | 0.0000         | 0.0059       | 0.0000       | 0.3630       | 0.1009       | 0.3666       | 0.0001      | 0.0163        |              | 0.0000          | 0.3627      |
| <i>rad51ap1</i> | 113  |     | 1.0000       | 0.0000      | 0.0000        | 0.0000         | 0.0046       | 0.0000       | 0.5030       | 0.0780       | 0.5001       | 0.0006      | 0.0113        | 0.0000       |                 | 0.2337      |
| <i>sfr1</i>     | 118  |     | 1.0000       | 1.0000      | 1.0000        | 1.0000         | 1.0000       | 1.0000       | 1.0000       | 1.0000       | 1.0000       | 1.0000      | 1.0000        | 1.0000       | 1.0000          |             |

