**Supplementary file 1:** Paleobiogeographic events used to constrain the age of 17 nodes in the phylogeny of the Aselloidea Latreille, 1802. <https://doi.org/10.5852/ejt.2024.968.2733.12545>.

JUGOVIC, J., MALEK-HOSSEINI, M.J., ISSARTEL, C., KONECNY-DUPRE, L., KUNTNER, M., FATEMI, Y., FLOT, J.-F., DOUADY, C.J., & MALARD, F. A second species of *Stenasellus* Dollfus, 1897 (Isopoda, Stenasellidae) from sulfidic groundwater of Iran described using morphological and molecular methods.

Saclier et al. (2024) used well-identified paleobiogeographic events as calibration points to constrain the age of 17 nodes in the phylogeny of the Aselloidea (Isopoda, Pancrustacea). We provide below a detailed description of these calibration points: they span a period ranging from 300 to 2 Myr before present. Divergence times among Aselloidea were estimated with PhyloBayes using a CAT-GTR + G + I model, the 17 calibration points as soft bounds, a birth–death prior on divergence time and a log-normal autocorrelation of the substitution rates among branches (Lepage et al., 2007). Saclier et al. (2024) tested the effect of removing any given calibration

point on divergence time estimates. They found that divergence time estimates were robust to the removal of any single palaeobiogeographic calibration point, except the deepest one that constrained the divergence between Stenasellidae and Asellidae to be more recent than 300 Myr. Removing this calibration point pushed back the divergence between the Stenasellidae and Asellidae to 300 Myr (95% credibility interval [CI]: 415-222 Myr) instead of 139 Myr (CI: 174-106 Myr), when including it.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Node | Point number | Species 1 | Species 2 | Maximum time (Myr) | Minimum time (Myr) | Event |
| Root | 16 | *Stenasellus nuragicus* (50) | *Proaellus* sp. (Hfeletto) (300) | 300 | NA | Phylipps et al. (2013) dated the divergence between Ligiiae and Oniscidea (two isopods groups) as more recent than the oldest known isopod fossil *(Hesslerella)* dated 300 Myr before present (BP). The divergence between these two groups is older than the divergence between Stenasellidae and Asellidae (Lins *et al.* 2012). Thus, the divergence between Stenasellidae and Asellidae is more recent than 300 Myr. |
| MRCA1 of *Gallasellus* (France) and *Conasellus* (US) | 13 | *Gallasellus heilyi* (408) | *Conasellus kenki* (369) | 300 | 54 | The genus *Gallasellus,* endemics to France, is sister to the genus *Conasellus,* endemics to the United States. The divergence between these two genera should be older than the last contact between the American and European continents, which dates back to 54 Myr BP (Skogseid *et al.* 2000). |
| Node | Point number | Species 1 | Species 2 | Maximum time (Myr) | Minimum time (Myr) | Event |
| MRCA of Corsican-Sardinian *Stenasellus* and Continental *Stenasellus* | 17 | *Stenasellus buili* (21) | *Stenasellus racovitzai* (23) | 250 | 29 | The distribution of Stenasellidae suggests a Neo-Tethys origin (see figure by Magniez (2008), in Malard e*t al.*,2014). The opening of Neo-Tethys is dated 250 Myr BP (Stampfli, 2000). Furthermore, the divergence between the Corsican and continental species of Stensallidae cannot be subsequent to the geological separation of the islands of Corsica-Sardinia from the continent, dated 29 Myr BP (Orsini *et al.*,1980). Thus, the divergence between Sardinian stenasellids *(Stenasellus racovitzai* MOTUs 23 & 46) and their continental relatives (*S. virei* MOTUs 45 & 47 and *S. buili* MOTU 21) should be dated between -250 and -29 Myr BP. |
| MRCA of *Bragasellus lagari* | 12 | *Bragasellus lagari* (86) | *Bragasellus lagari* (84) | 75 | NA | The *Bragasellus lagari* (MOTUs 81–87) clade is distributed in a restricted area, which was marine until 75 Myr BP (Tyson and Funnell, 1987; Dercourt *et al.*,2000). |
| MRCA of five clades | 1 | *Proasellus* sp. (Santa Anna) (301) | *Proasellus* sp.(Tende) (310) | 70 | NA | Four clades belonging to the Alpine *Proasellus* and one clade belonging to the *anophtalmus* lineage are distributed in areas which were marine until 70 Myr BP (Tyson and Funnell, 1987; Kędzierski and Leszczyński, 2013). |
| 2 | *Proasellus cavaticus* (295) | *Proasellus walteri* (312) |
| 3 | *Proasellus* sp. (Montelimar) (311) | *Proasellus synaselloides* (277) |
| 4 | *Proasellus* sp. (Chamois) (280) | *Proasellus* sp. (Martino) (285) |
| 6 | *Proasellus hercegovinensis* (220) | *Proasellus anophtalmus* (216) |
| Node | Point number | Species 1 | Species 2 | Maximum time (Myr) | Minimum time (Myr) | Event |
| MRCA of *Proasellus* species of the Asón valley (Spain) | 11 | *Proasellus chappuisi* (120) | *Proasellus ortizi* (128) | 54 | NA | All *Proasellus* species from the Asón valley, Spain (*P. cantabricus*, *P. chappuisi*, *P. ebrensis*, *P. grafi,* *P. ortizi*, *P.* sp. (Cuvera2) and *P. stocki*) constitute a clade distributed in a restricted area which was marine until 54 Myr BP (Payros et al., 2006; Orue-Etxebarria et al., 2001; Baceta *et al.*, 2004). |
| MRCA of *Proasellus.of* the Basque region | 10 | *Proasellus* sp. (Aquerreta) (151) | *Proasellus aragonensis* (153) | 49 | NA | *Proasellus* species of the Basque Region (*P. aquacecalidae*, *P. alavensis*, *P. aragonensis*, *P. guipuzcoensis*, *P.* sp. (Aquerreta), *P.* sp. (Etcheberrigaray), *P. spelaeus* and *P. vandeli*) occupy an area, which was marine until 49 Myr BP (Plaziat, 1981, Pujalte *et al.*,2000; Baceta *et al.*, 2004). |
| MRCA of Corsican-Sardinian *Proasellus* and Continental *Proasellus* | 7 | *Proasellus ibericus* (178) | *Proasellus ligusticus* (192) | NA | 29 | The divergence between Corsican and continental species of *Proasellus* cannot be subsequent to the geological separation between the islands of Corsica-Sardinia from the continent, dated 29 Myr BP (Orsini *et al.*,1980). Thus, Corsican and Sardinian *Proasellus* (clade including *P. beroni* (188-190) and *P. ezzu* and *P. ruffoi* (104-108)) should have diverged from their continental relatives (clade including *P. rectus* (166)) over 29 Myr BP. |
| MRCA of *Mesoasellus* and *Limnoasellus* (Baikal Lake) | 15 | *Mesoasellus dybowskii* (53) | *Limnoasellus poberezhnii* (54) | 27 | NA | The divergence between closely related species endemic to the Lake Baikal (Russia) should not be older than the birth of the lake dated 27 Myr BP (Petit and Déverchère, 2006, Grabowski et al., 2017). Thus, divergence between *Mesoasellus dybowskii* and *Limnoasellus poberezhnii* (*nomen nudum*) as well as divergence between the two *Baicalasellus* species should be younger than 27 Myr BP. |
| MRCA of *Baicalasellus* species (Baikal Lake) | 14 | *Baicalasellus baicalensis* (413) | *Baicalasellus korotnevi* (412) |
| Node | Point number | Species 1 | Species 2 | Maximum time (Myr) | Minimum time (Myr) | Event |
| MRCA of betic *Proasellus* species | 8 | *Proasellus* aff. *escolai* (179) | *Proasellus comasi* (172) | 19 | NA | All Betic *Proasellus* species (*P*. aff. *escolai,* *P.* aff. *lagari,* *P. bellesi*, *P.* *beticus*, *P. bouianus*, *P. comasi,* *P. escolai*, *P. espanoli*, *P. gourbaultae*, *P. granadensis*, *P. jaloniacus*, P*. lagari,* *P. meijersae*, *P. solanasi* and *P.* sp. (Lentegi)) constitute a clade distributed in a restricted area which was a marine area until 19 Myr BP (Meulenkamp and Sissingh, 2003). Considering a complete clade migration as unlikely, we can expect that this clade diversified in this area after marine regression, allowing us to make the assumption that this clade is younger than 19 Myr BP. |
| MRCA of Cretan *Proasellus* | 5 | *Proasellus (coxalis)* sp. (Mesklasp) (253) | *Proasellus minoicus* (255) | 17 | NA | Cretan species of *Proasellus* probably diversified after the island emerged from the sea 17 Myr BP (Rögl, 1988; Welter-Schultes, 2000). |
| Node | Point number | Species 1 | Species 2 | Maximum time (Myr) | Minimum time (Myr) | Event |
| MRCA of *Proasellus* of Ohrid Lake, Macedonia | 9 | *Proasellus remyi* (132) | *Proasellus gjorgjevici* (133) | 2 | NA | *Proasellus arnautovici*, *P. gjorgjevici* and *P. remyi* are endemic to the Lake Ohrid (border between Albania and North Macedonia). These species constitute two lineages, one of which is found in deep layers of the lake, whereas the other is found in the shallow layers, suggesting an intralacustrine speciation (Wysocka *et al.,* 2008). The divergence between these two lineages should be younger than the origin of the lake, dated 2 Myr BP maximum (1.3 Myr to 1.9 Myr in Wagner et al., 2017; >1.2 Myr in Wagner et al., 2014 and 2 Myr in Stelbrink et al., 2016 and in Lindhorst et al., 2015). |

1 MRCA: most recent common ancestor

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