Phylogeny of the genus *Percus* (Coleoptera: Carabidae) – nuclear genes and the basal splits

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Brückner, M. & Mossakowski, D. 2006: Phylogeny of the genus *Percus* (Coleoptera: Carabidae) – nuclear genes and the basal splits. — Entomol. Fennica 17: 195–199.

The phylogeny of the western Mediterranean genus *Percus* s.l. (Coleoptera, Carabidae) was analysed using partial DNA sequences of the nuclear 28S rRNA gene (865 bp). All 18 species of *Percus* s.l. with exception of *P. espagnoli* from the Balearic Islands were included. Phylogenetic analysis using the Maximum likelihood method reveals that the genus splits into three groups. The French *Percus villai* stands on its own. The Tyrrhenian species of Corsica, Sardinia, Sicily and the Italian mainland form the second group. Within this group, the phylogenetic relationships are not resolved. The third group includes *Percus plicatus* from Mallorca and the species of the subgenus *Pseudopercus* from the Iberian Peninsula. These results indicate that the subgenus *Percus* s.str. is paraphyletic. The split of *Pseudopercus* and *P. plicatus* probably occurred with the separation of the Balearic islands from the Iberian peninsula (20 million years ago) or by the flooding of the Mediterranean after the Messinian salinity crisis (5.3 million years ago). Based on these assumptions, the divergence rate of the 28S gene can be estimated as being at 0.22–0.27% or at 0.99–1.01% per million years.

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Received 7 December 2005, accepted 08 June 2006

1. Introduction

The genus *Percus* s.l. (Coleoptera: Carabidae) consists of 18 species which are distributed in the western Mediterranean area (Fig. 1). Traditionally this genus was divided into two subgenera, *Percus* s.str. and *Pseudopercus*. The 14 species of *Percus* s.str. are distributed around the Tyrrhenian Sea (Corsica, Sardinia, Sicily and the Italian mainland), in the French Maritime Alps (*Percus villai*) and in the Balearic Islands (*P. plicatus* in Mallorca and Menorca and *P. espagnoli* in Cabrera and Foradada). The subgenus *Pseudopercus* consists of four species which are distributed in the south-eastern part of the Iberian Peninsula.

The distinction between *Pseudopercus* and *Percus* s.str. is based mainly on the keel at the 7th interstriae which is more distinct in *Percus* s.str. than in *Pseudopercus* (Lagar Mascaro 1965). Ganglbauer (1909) and Porta (1923) divided the *Percus* species using the *series umbilicata* at the 8th interstriae which is missing in *Pseudopercus* and in *P. strictus*, *P. grandicollis* and *P. cylindricus*. Enlarged and transversally furrowed temples are present in *P. strictus*, *P. grandicollis* and

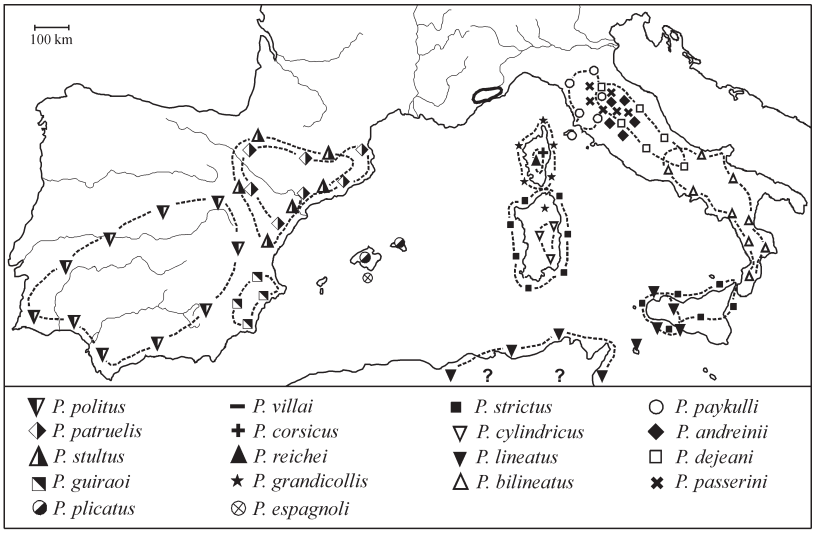

Fig. 1. Distribution of the *Percus* s.l. species; modified after Lagar Mascaro (1965) and Casale & Vigna Taglianti (1982).

P. corsicus. Ganglbauer (1909) described a close ("sehr nahe") relationship of *P. passerini*, *P. villai*, *P. lineatus* and *P. bilineatus* which all show striae on the elytra. However, a morphologic analysis of the genus *Percus* s.l. based on the principles of Phylogenetic Systematics is missing up to now.

A detailed analysis of the species complex of *P. strictus* and *P. grandicollis* was performed by Brückner (2001, 2002a) using mtDNA sequences of protein coding genes, RAPD and morphometric data. An allozyme analysis of *P. strictus* from Sardinia was performed by Ketmaier *et al.* (2003). Phylogenetic analyses of the *Pseudopercus* species based on mitochondrial and nuclear DNA sequences lead to different gene trees which must be the result of ancestral polymorphism or introgression (Brückner 2004). A detailed phylogenetic and biogeographic analysis was performed by Brückner (2002b). In the present study, the monophyly of the two subgenera and the phylogeny of the basal groups are analysed using nuclear gene sequences.

DNA sequences of slow evolving nuclear genes like the 28S rRNA gene seem to be suitable especially to resolve deep branches and older splits in carabids, since this gene has been successfully used for phylogenetic analyses in insects and especially in carabids before (Friedrich & Tautz 1995, 1997, Kim *et al.* 2000). In *Pseudopercus* species, the 28S rRNA gene evolves 2–3 times slower than mitochondrial genes (Brückner 2004). A further advantage of nuclear rRNA genes is their concerted evolution (Zimmer *et al.* 1980). The intraspecific variability of this gene is low, so phylogenetic analyses can be performed with only few individuals of each species for an appropriate phylogenetic reconstruction (e.g., Hillis & Dixon 1991).

2. Material and methods

DNA sequences of all species of the genus *Percus* s.l. (except for *Percus espagnoli*) from the following collection sites were analysed: *P. passerini* (Dejean, 1828) (Tuscany, Italy), *P. paykulli* Rossi, 1790 (Tuscany, Italy), *P. andreinii* Mainardi, 1914 (Umbria, Italy), *P. dejeani* Dejean, 1831 (Lazio and Marche, Italy), *P. bilineatus* (Dejean, 1828) (Calabria, Italy), *P. reichei* Kraatz, 1858 (Corsica, France), *P. corsicus* Serville, 1820 (Corsica, France), *P. grandicollis* Serville, 1820 (Corsica, France), *P. strictus* Dejean, 1828 (Sardinia and Sicily, Italy) and *P. cylindricus* Chaudoir, 1868 (Sardinia, Italy). DNA sequences are deposited in the GenBank data base under accession numbers DQ789060–DQ789074. The following sequences (Acc. No. AY334311–AY334317) were obtained from a previous study (Brückner 2004): *Percus villai* Kraatz, 1858 (Maritime Alps, France), *P. lineatus* Solier, 1835 (Sicily, Italy), *P. plicatus* (Dejean, 1828) (Mallorca, Spain), *Pseudopercus politus*

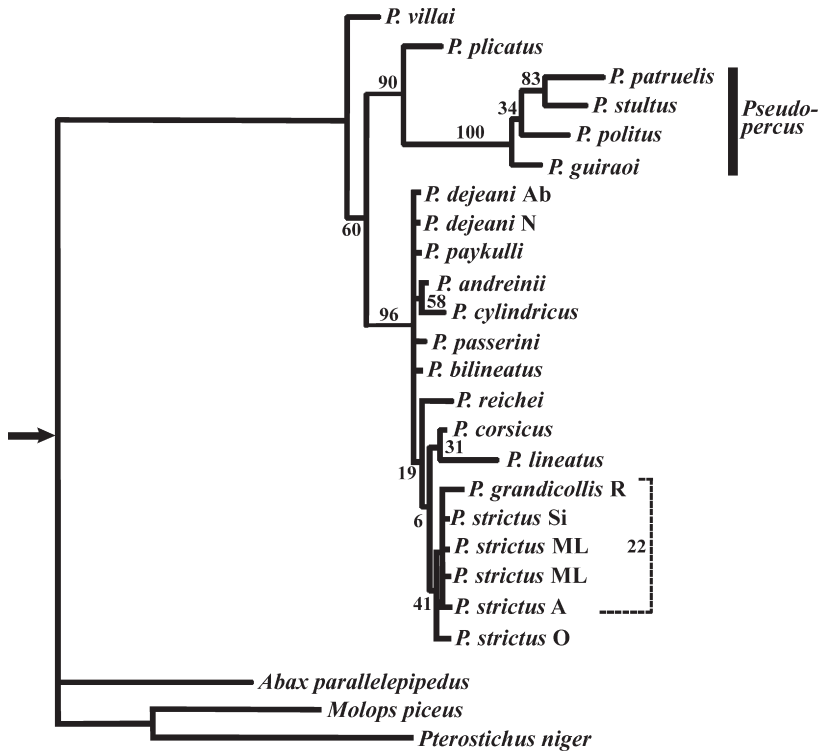

Fig. 2. Maximum Likelihood tree of the 28S rRNA data set (–ln likelihood = 3130.09). Abbreviations: A: Arbatax, O: Oristano, ML: M. Limbara (all Sardinia, Italy), Si: Sicily (Italy), R: Gorges de la Restonica (Corsica, France), Ab: Abruzzi, N: M. Nerone, Marche (both Italy). Bootstrap values are given at the branches. Branch lengths are proportional to the number of substitutions.

(Dejean, 1831) (Andalusia, Spain), *Pseudopercus patruelis* Dufour, 1820 (Girona, Spain), *Pseudopercus guiraoi* Perez-Arcas, 1869 (Murcia, Spain) and *Pseudopercus stultus* Dufour, 1820 (Castellón, Spain).

DNA was isolated from thoracic muscle of frozen specimen using the QIAamp tissue kit (Qiagen). The amplification of the analysed part of the 28S rRNA gene (approx. 1,000 basepairs) was performed using the primers 28S-01 and 28SR-01 (Kim *et al.* 2000) (PCR cycles: 5 min 96 °C (1x); 90 sec 96 °C, 90 sec 46 °C, 90 sec 68 °C (5x); 90 sec 96 °C, 90 sec 50 °C, 90 sec 68 °C (28x); 3 min 68 °C). After separation on an agarose gel and a cleaning step using the Qiaex II gel extraction Kit (Qiagen), DNA sequencing of the PCR product was done on an ABI 373A sequencer using the PCR primers. In addition, two internal primers were designed for sequencing (28S-i: 5'-GTT TAC CCC TGA ACA GTT TCA CG-3'; 28S-ir: 5'-GTG AAA CTG TTC AGG GGT AAA CC-3').

For alignment the ClustalX program (Thompson *et al.* 1997) was used (gap opening: 10; delay sequence divergence: 40%; DNA transition weight: 0.5). Gaps and parts of uncertain homology were removed from the data set. Homoplasy content was checked using transition-transversion ratio and skewness. Phylogenetic analysis was performed using the Maximum Likelihood (ML) algorithm in PAUP* 4.0b10 (Swofford 1998). The HKY85 evolution model with empirical base frequencies and observed transition/transversion ratio was chosen for the analysis.

Branch support was checked by bootstrap analysis (1,000 pseudoreplicates). Two further Molopini, viz. *Abax parallelepipedus* and *Molops piceus*, and one Pterostichini, viz. *Pterostichus niger*, were chosen as out-group species for the present analysis.

3. Results

After removing gaps and parts of uncertain homology, the 28S rRNA data matrix consists of 865 positions in 25 taxa. The 28S rRNA gene GC content (42.4%) is much higher than in insect mitochondrial genes. The data set of the in-group

contains 107 variable and 57 phylogenetically informative sites. The highest observed p-distance within the in-group is 7.13% (*P. patruelis* and *P. cylindricus*). The mean p-distance is 2.78% within the in-group. The high transition-transversion ratio (1.73) and the low g_1 value (–1.56) indicate a low homoplasy content in the 28S rRNA data set so that the phylogenetic results are probably based on synapomorphic characters.

The phylogenetic analysis of the 28S rDNA sequences resulted in the Maximum Likelihood tree shown in Fig. 2. Three groups are found: *Percus villai* stands on its own. The Tyrrhenian species form a second group, supported by 96% bootstrap value. The Spanish *Pseudopercus* species and *Percus plicatus* from the Balearic Islands build up the third group which is supported by 90% bootstrap value. Considering these three groups *P. villai* splits first.

4. Discussion

The basal split between *P. villai* and all other investigated in-group species is only weakly supported. A bootstrap value of 60% is too low to discuss this basal split as the most ancient speciation process occurred in the genus *Percus* s.l. Therefore, the relationship of the three major groups remains unresolved.

The Tyrrhenian group is supported by the high bootstrap value of 96%. However, the splits within this monophyletic group are much more recent than the other splits because of the very short branch lengths. Therefore, no resolution is found using the slowly evolving 28S rDNA sequences. Other genes with higher substitution rates such as mitochondrial genes are more successful in resolving these relationships (Brückner 2002).

The four *Pseudopercus* species form a monophyletic group which is supported by a 100% bootstrap value. This subgenus is sister to *P. plicatus* (subgenus *Percus*) from Mallorca with a 90% bootstrap support. This split between *P. plicatus* and the ancestor of the *Pseudopercus* species was probably caused by the separation of the Balearic Islands from the Iberian Peninsula which was dated back to approx. 20 myr ago (Rögl & Steininger 1983). Alternatively, this split could have happened at the end of the Messinian (5.3 million years ago) when the Mediterranean was flooded again after a period of drying (Hsü *et al.* 1977). The observed genetic distances (percentage of divergence) of the 28S DNA sequences between *P. plicatus* and *Pseudopercus* are 4.32–5.36%. Based on these data and assuming a constant substitution rate at each branch, the divergence rate of the 28S gene can be calculated at 0.22–0.27% or at 0.99–1.01% per million years.

The positioning of *P. plicatus* together with *Pseudopercus* indicates that the subgenus *Percus* s.str. is not monophyletic and it is divided in the three major groups mentioned above. The main morphological character which differentiates the two subgenera is the keel at the basis of the 7th interstriae which is more distinct in *Percus* s.str. than in *Pseudopercus*. The phylogenetic results indicate that the distinct keel of the *Percus* s.str. species is the plesiomorphic character state within *Percus* s.l. Within the other Molopini, the genus *Abax* shows a distinct keel, but *Molops* does not. So the interpretation of the distinct keel as the apomorphic or plesiomorphic character state of the Molopini is difficult. Following the result of a phylogenetic analysis of the genera *Abax*, *Molops* and *Percus* which resulted in a sistergroup relationship of *Abax* and *Molops* (Düring & Brückner 2000), the keel of *Abax* and *Percus* can be interpreted as convergent. The other possibility is that the keel is the plesiomorphic character state within the Molopini and has been lost in *Molops*. Other traits like the shape of pronotum, the basal fovea of the pronotum and the form of the elytrae are not very well suited for differentiation of the two subgenera because of the high morphologic variability within some *Percus* s.str. species, e.g., in *P. strictus*.

In conclusion, the division of *Percus* s.l. into two monophyletic subgenera is refuted.

Further investigations using morphological characters of the paraphyletic *Percus* s.str. species should lead to a revision of the genus.

Acknowledgements. We would like to thank P. Brandmayr, K. Desender, A. Düring, J. Galián, B. Klein, H. Knitter, J.-C. Malausa, M. Marciniak, A. Ortiz, F. Prüser and J. Serrano for providing beetle specimens, and A. Düring for helpful discussion.

References

Brückner, M. 2001: Inter- and Intrapopulation Differentiation in *Percus strictus* and *Percus grandicollis* – RAPD-Analysis in two closely related carabid species. — Mitt. Dtsch. Ges. Allg. Angew. Ent. 13: 95–100.

Brückner, M. 2002a: Out of Sardinia – Cladistic biogeographic analysis of *Percus strictus* and *Percus grandicollis*. — In: Szyszko, J. *et al.* (eds.), How to protect or What we know about Carabid Beetles, X European Carabidologists Meeting, Tuczno 2001, Poland: 321–329. Warsaw Agricultural Press.

Brückner, M. 2002b: Phylogenie und Biogeographie der Gattung *Percus* Bonelli 1810 (Coleoptera, Carabidae): Eine molekularsystematische Analyse. — Academic thesis, University of Bremen. 245 pp.

Brückner, M. 2004: The phylogeny of the subgenus *Pseudopercus* (Coleoptera, Carabidae): Conflicts between mitochondrial and nuclear DNA sequences. — Mitt. Dtsch. Ges. Allg. Angew. Ent. 14: 77–82.

Casale, A. & Vigna Taglianti, A. 1982: I Coleotteri Carabidi delle Alpi Liguri: composizione della fauna ed origine del popolamento. — Lavori della Societa' Italiana di Biogeografia 9: 567–598. [In Italian.]

Düring, A. 2004: Molekularsystematische Untersuchungen am Carabidentaxon *Abax* Bonelli 1810 (Coleoptera, Carabidae). — Academic thesis, University of Bremen. 363 pp.

Düring, A. & Brückner, M. 2000: The evolutionary history of the tribe Molopini: A first molecular approach. — In: Brandmayr, P. *et al.* (eds.), Natural History and Applied Ecology of Carabid Beetles – Proceedings of the IX European Carabidologists' Meeting (26–31 July 1998, Camigliatello, Cosenza, Italy): 1–4. Pensoft, Sofia, Moskow.

Friedrich, M. & Tautz, D. 1995: Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. — Nature 376: 165–167.

Friedrich, M. & Tautz, D. 1997: Evolution and phylogeny of the Diptera: A molecular phylogenetic analysis using 28S rDNA sequences. — Syst. Biol. 46 (4): 674–698.

Ganglbauer, L. 1909: *Percus*-Studien. — Deutsch. Ent. Zeitschr. 1: 97–105.

Hillis, D. M. & Dixon, M. T. 1991: Ribosomal DNA: Molecular evolution and phylogenetic inference. — The Quarterly Review of Biology 66 (4): 411–453.

Hsü, K. J., Montadert, L., Bernoulli, D., Cita, M. B., Erickson, A., Garrison, R. E., Kidd, R. B., Mèlierés, F., Müller, C. & Wright, R. 1977: History of the Mediterranean salinity crisis. — Nature 267: 399–403.

Ketmaier, V., Casale, A., Cobolli, M., de Matthaeis, E. & Vigna Taglianti, A. 2003: Biochemical systematics and phylogeography of the *Percus strictus* subspecies (Coleoptera, Carabidae), endemic to Sardinia. — Ital. J. Zool. 70: 339–346.

Kim, C.-G., Zhou, H.-Z., Imura, Y., Tominaga, O., Su, Z.-H. & Osawa, S. 2000: Pattern of morphological diversification in the *Leptocarabus* ground beetles (Coleoptera: Carabidae) as deduced from mitochondrial ND5 gene and 28S rDNA sequences. — Mol. Biol. Evol. 17(1): 137–145.

Lagar Mascaro, A. 1965: Revisión de las especies iberobaleares del genero "*Percus*" Bonelli ("Coleoptera Pterostichidae"). — Miscelanea Zoologica 21: 79–88. [In Spanish.]

Nei, M. & Kumar, S. 2000: Molecular evolution and phylogenetics. — Oxford University Press, New York. 333 pp.

Porta, A. 1923: Fauna Coleopterorum Italica Vol. I – Adephaga. — Stabilimento Tipografico Piacentino, Piacenza. [In Italian.]

Rögl, F. & Steininger, F. F. 1983: Vom Zerfall der Tethys zu Mediterran und Paratethys. — Ann. Naturhist. Mus. Wien 85 A: 135–163.

Swofford, D. L. 1998: PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), Version 4. — Sinauer Associates, Sunderland, Massachusetts.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. — Nucleic Acids Res. 24: 4876–4882.

Zimmer, E. A., Martin, S. L., Beverley, S. M., Kan, Y. W. & Wilson, A. C. 1980: Rapid duplication and loss of genes coding for the a chains of hemoglobin. — Proc. Natl. Acad. Sci. USA 77 (4): 2158–2162.