



Molecular systematics and morphological identification of the cryptic species of the genus *Acalles* Schoenherr, 1825, with descriptions of new species (Coleoptera: Curculionidae: Cryptorhynchinae)

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Abstract

Molecular systematics and morphological study of the monophyletic weevil genus *Acalles* Schoenherr, 1825 are presented. Based on the mitochondrial CO1 barcoding gene and 16S ribosomal RNA gene, we discuss three difficult species complexes in the framework of a molecular phylogenetic reconstruction of 37 of 47 Western Palaearctic *Acalles* species or subspecies: the *A. echinatus*, *A. maraoensis* and *A. sierrae* complexes. Two results are given: 1. An exclusive focus on morphological, exoskeletal methods reach their limits in the case of many cryptic Cryptorhynchinae. In these cases molecular analysis is indispensable to resolve species level questions. 2. By using a combination of phenotypic and genotypic characters it is not only possible to ascertain phylogenetic relationships, but also to uncover new morphological, non-intraspecific characteristics. Digital photography with image stacking makes this possible: for the first time we present photo key for *Acalles* species, a reliable, less costly and quick method for identification alongside DNA barcoding. The following taxonomic changes are given: *Coloracalles edoughensis* Desbrochers, 1892 **comb. nov.** (formerly *Acalles edoughensis*) from North Africa and Spain change to *Coloracalles* Astrin & Stüben, 2008 and *Pseudodichromacalles xe-*

structure of the aedeagus which is characteristic of *Acallobrates* (compare with fig. 24, 25 and Bahr 2003). The above mentioned suspicion based on morphological characters has now been confirmed by molecular analysis.

However, these first impressions and results that we have stated here we hope to expand upon in the upcoming years in the M.W.I. Project (Molecular Weevil Identification). For this task, we ask colleagues to send us determined specimens in ethanol from all over the world, from as many Cryptorhynchinae genera as possible.



FIGURES 24–25. *Acallobrates minutesquamosus* from Europe, habitus and aedeagus.

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References

- Astrin, J.J. & Stüben, P.E. (2008) Phylogeny in cryptic weevils: molecules, morphology and new genera of Western Palearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics*, 22, 503–522.
<http://dx.doi.org/10.1071/is07057>
- Astrin, J.J. & Stüben, P.E. (2009) Molecular phylogeny in 'nano-weevils': description of a new subgenus and two new species of *Calacalles* from the Macaronesian Islands (Curculionidae: Cryptorhynchinae). *Zootaxa*, 2300, 51–67.
- Astrin, J.J. & Stüben, P.E. (2010) Molecular phylogeny of *Echinodera* and *Ruteria* (Coleoptera: Curculionidae: Cryptorhynchinae) and the parallel speciation of Canary Island weevils along replicate environmental gradients. *Invertebrate Systematics*, 24, 434–455.
<http://dx.doi.org/10.1071/is10021>
- Astrin, J.J., Stüben, P.E., Misof, B., Wägele, J.W., Gimmich, F., Raupach & M.J., Ahrens, D. (2012) Exploring diversity in cryptorhynchine weevils (Coleoptera) using distance-, character- and tree-based species delineation. *Molecular Phylogenetics and Evolution*, 63, 1–14.
<http://dx.doi.org/10.1016/j.ympev.2011.11.018>
- Bahr, F. & Stüben, P.E. (2002) Digital-Weevil Determination—Transalpina: *Cryptorhynchinae*. (Coleoptera: Curculionoidea). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 3, 14–87.
- Bahr, F. (2003) Revision des Genus *Acallobrates* Reitter, 1913 (Coleoptera: Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 4 (37), 101–115.
- Bourke, B.P., Oliveira, T.P., Suesdek, L., Bergo, E.S. & Sallum, M.A.M. (2013) A multi-locus approach to barcoding in the *Anopheles strodei* subgroup (Diptera: Culicidae). *Parasites & Vectors*, 6 (1), 111.
<http://dx.doi.org/10.1186/1756-3305-6-111>

- Buse, J. (2012) "Ghosts of the past": flightless saproxylic weevils (Col.: Curculionidae) are relict species in ancient woodlands. *Journal of Insect Conservation*, 16, 93–102.
<http://dx.doi.org/10.1007/s10841-011-9396-5>
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540–552.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334>
- Crandall, K.A. & Fitzpatrick, J.F. (1996) Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology*, 45 (1), 1–26.
<http://dx.doi.org/10.1093/sysbio/45.1.1>
- CURCULIO Team (2006) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Sitona* (Entiminae: Sitonini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 7 (85), 14–20.
- CURCULIO Team (2007) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Baris / Limnobaris* (Baridinae: Baridini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 8 (97), 12–18.
- CURCULIO Team (2008) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. *Acalyptus / Ellescus / Dorytomus* (Curculioninae: Acalyptini & Ellescini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 9 (107), 11–18.
- CURCULIO Team (2009) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. *Isochnus / Orchestes / Pseudorchestes / Rhamphus / Rhynchaenus / Tachyerges* (Curculioninae: Rhamphini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 10 (119), 13–25.
- CURCULIO Team (2010) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Tychius* (Curculioninae: Tychiini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 11 (149), 27–39.
- CURCULIO Team (2011) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Polydrusus* (Entiminae: Polydrusini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 12 (173), 11–24.
- Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, 85, 407–415.
<http://dx.doi.org/10.1111/j.1095-8312.2005.00503.x>
- Dieckmann, L. (1982) *Acalles*-Studien (Coleopter, Curculionidae). *Entomologische Nachrichten und Berichte*, 26, 195–209.
- East-CURCULIO Team (2010) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Brachypera / Donus* (Hyperinae: Hyperini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 11 (151), 102–108.
- East-CURCULIO Team (2011) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Bagous* (Bagoinae: Bagoini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 12 (175), 39–56.
- East-CURCULIO Team (2012) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Rhynchitidae & Attelabidae. *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 13 (193), 138–161.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.
- Klausnitzer, B. (2010) Entomologie—quo vadis? *Nachrichtenblatt der Bayerischen Entomologen*, 59 (3/4), 99–111.
- Lachowska, D., Rozek, M. & Holecova, M. (2009) Chromosomal similarities and differences among three sibling species of the *Acalles echinatus* group (Coleoptera, Curculionidae, Cryptorhynchinae). *Zootaxa*, 1985, 63–68.
- Lanave, C., Preparata, G., Saccone, C. & Serio, G. (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution*, 20 (1), 86–93.
- Padial, J.M., Castroviejo-Fisher, S., Köhler, J., Vilà, C., Chaparro, J.C. & Riva, I.D.I. (2009) Deciphering the products of evolution at the species level: the need for an integrative taxonomy. *Zoologica Scripta*, 38, 431–447.
<http://dx.doi.org/10.1111/j.1463-6409.2008.00381.x>
- Papp, C.S. (1979) *An illustrated catalog of the Cryptorhynchinae of the New World. With generic descriptions, references to the literature and deposition of the type material (Coleoptera: Curculionidae)*. Department of Food and Agriculture, State of California, 467 pp.
- Rambaut (2012) molecular evolution, phylogenetics and epidemiology, FigTree 1.3.1. Available from <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 24th Jan 2012)
- Riedel, A., Sagata, K., Shardjono, Y.R., Tänzler, R. & Balke, M. (2013) Integrative taxonomy on the fast track – towards more sustainability in biodiversity research. *Frontiers in Zoology*, 10, 1–15.
<http://dx.doi.org/10.1186/1742-9994-10-15>
- Rheinheimer, J. (2007) Neue Rüsselkäfer aus dem tropischen Amerika (Col.: Apionidae, Curculionidae). *Koleopterologische Rundschau*, 77, 255–262.
- Rheinheimer, J. (2008) Neue Rüsselkäfer aus Französisch Guayana (Col.: Curculionidae). *Koleopterologische Rundschau*, 78, 419–428.
- Rheinheimer, J. & Hassler, M. (2010) *Die Rüsselkäfer Baden-Württembergs*. Verlag Regionalkultur, Heidelberg-Ubstadt, Weiher-Neustadt a.d.W., Basel, 944 pp.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12), 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Skuhrovec, J. (2009) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Hypera/Limobius/*

- Metadonus* (Hyperinae: Hyperini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 10 (121), 39–47.
- Skuhrovec, J., Caldara, R., Stejskal, R., Bahr, F., Trnka, F. & Gosik, R. (2013) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Brachycerinae (Brachycerini, Erihrinini & Tanysphyrini). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 14 (215), 1–17.
- Solari, A. & Solari, F. (1907) Studi sugli *Acalles*. In: Doria, G. (Eds.), *Annali del Museo Civico di Storia Naturale*, Series 3, 3 (43), 479–551. [Sep. pp. 1–73]
- Sprick, P. & Stüben, P.E. (2000) Ökologie der kanarischen Cryptorhynchinae außerhalb des Laurisilva (Coleoptera, Curculionidae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 1, 318–341.
- Stüben, A. (2011) Schichtfotographie in der Entomologie. *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 12 (187), 1–13. [reprint]
- Stüben, P.E. & Behne, L. (1998) Revision der *Acalles krueperi*-Gruppe mit Beschreibung der Gattung *Dichromacalles* g. n. und der Untergattung *Balcanacalles* subg. n. (Coleoptera, Curculionidae, Cryptorhynchinae). *Entomologische Blätter*, 94, 11–32.
- Stüben, P.E. (1998) Die südeuropäischen Arten der Gattungen *Echinodera* Wollaston und die Gattung *Ruteria* Roudier stat.n. (Coleoptera: Curculionidae: Cryptorhynchinae). *Beiträge zur Entomologie*, 48 (2), 417–448, Berlin.
- Stüben, P.E. (1999a) Taxonomie und Phylogenie der westpaläarktischen Arten der Gattung *Kyklioacalles* g.n. (Coleoptera, Curculionidae: Cryptorhynchinae)—*Stuttgarter Beiträge zur Naturkunde*, Series A, Nr. 584, 38. [S. Stuttgart]
- Stüben, P.E. (1999b) Die westpaläarktischen Arten der Gattung *Onyxacalles* g. n. (Coleoptera: Curculionidae, Cryptorhynchinae). *Entomologische Blätter*, 95, 175–203. [Jena]
- Stüben, P.E. (2000a) Phylogenie der endemischen Taxa des Genus *Acalles* von den Kanarischen Inseln (Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 1, 287–292.
- Stüben, P.E. (2000b) Biogeographie und Evolution der kanarischen Cryptorhynchinae (Curculionidae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 1, 293–306.
- Stüben, P.E. (2000c) Die Arten des Genus *Acalles* von den Kanarischen Inseln (Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 1, 22–98.
- Stüben, P.E. (2003) Revision des Genus *Kyklioacalles* und Beschreibung der Untergattung *Palaeoacalles* subg. n. unter Heranziehung phylogenetischer, morphogenetischer und biogeographischer Aspekte (Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 4, 116–166.
- Stüben, P.E., Behne, L. & Bahr, F. (2003) Analytischer Katalog der westpaläarktischen Cryptorhynchinae/Analytical Catalogue of Westpalaearctic Cryptorhynchinae. Teil 2 / Part 2: *Acalles*, *Acallobrates* (Col.: Curculionidae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 4, 11–100.
- Stüben, P.E. (2004) Beschreibung neuer Arten aus der *Acalles sierrae*-Gruppe (Coleoptera: Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 5, 86–99.
- Stüben, P.E. (2005) "Basar Taxonomie"?—Ein erfolgreiches Kreuzungsexperiment zu *Acalles aeonii* Wollaston, 1864 (Coleoptera: Curculionidae). *Weevil News*, 31, 1–13. [ISSN: 1615-3472]
- Stüben, P.E. & Astrin, J.J. (2006) New insights from biogeography, morphology and molecular biology: the species status of *Acalles temperei* Péricart, 1987 and *Kyklioacalles navieresi* (Boheman, 1837) (Curculionidae: Cryptorhynchinae). *Weevil News*, 33, 1–8. [ISSN: 1615-3472]
- Stüben, P.E. & Astrin, J.J. (2009) New insights from biogeography, morphology and molecular biology: the species status of *Acalles temperei* Péricart, 1987 and *Kyklioacalles navieresi* (Boheman, 1837) (Curculionidae: Cryptorhynchinae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 10 (145), 217–223. [reprint of *Weevil News* article from 2006, see previous reference]
- Stüben, P.E. (2009) Neubeschreibungen west paläarktischer Cryptorhynchinae III—(Coleoptera: Curculionidae). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 10, 94–106.
- Stüben, P.E. & Astrin, J.J. (2010a) Molecular phylogeny in endemic weevils: revision of the genera of Macaronesian Cryptorhynchinae (Coleoptera: Curculionidae). *Zoological Journal of the Linnean Society*, 160, 40–87.
- Stüben, P.E. & Astrin, J.J. (2010b) Molecular phylogeny of the weevil genus *Kyklioacalles* Stüben, with descriptions of a new subgenus *Glaberacalles* and two new species (Curculionidae: Cryptorhynchinae). *Zootaxa*, 2662, 28–52.
- Stüben, P.E. & Astrin, J.J. (2012) Integrative Taxonomy, Phylogeny, and New Species of the Weevil Genus *Onyxacalles* Stüben (Coleoptera: Curculionidae: Cryptorhynchinae). *Psyche*, 2012, 1–22.
<http://dx.doi.org/10.1155/2012/654948>
- Stüben, P.E., Sprick, P., Müller, G., Bayer, Ch., Behne, L. & Krátký, J. (2012) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: Ceutorhynchinae (1. part). *Mononychini, Phytobiini, Hypurini, Cnemogonini, Scleropterini & Amalini*. *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 13 (192), 18–33.
- Stüben, P.E., Müller, G., Krátký, J., Bayer, Ch., Behne, L. & Sprick, P. (2013) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: Ceutorhynchinae (2. part). (Ceuthorhynchini: *Amalorrhynchus*, *Drupenatus*, *Poophagus*, *Coeliodes*, *Pseudocoeliodes*, *Coelioidinus*, *Eucoeliodes*, *Neoxyonyx*, *Thamiocolus*, *Micrelus*, *Zacladus*, *Phrydiuchus*, *Stenocarus*, *Nedyus*, *Ceutorhynchus*: Marklissus). *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 14 (210), 23 pp.
- Stüben, P.E., Schütte, A. & Astrin, J.J. (2013a) Molecular phylogeny of the weevil genus *Dichromacalles* Stüben (Curculionidae: Cryptorhynchinae) and description of a new species. *Zootaxa*, 3718 (2), 101–127.

<http://dx.doi.org/10.11646/zootaxa.3718.2.1>

- Stüben, P.E. & Schütte, A. (2013b) Die Wiederentdeckung von *Canariacalles xerampelinus* (Wollaston, 1864) und die Stellung im System der kanarischen Cryptorhynchinae (Curculionidae: Cryptorhynchinae), *Snudebiller, Studies on taxonomy, biology and ecology of Curculionoidea*, 14 (281), 1–14.
- Sudhaus, W. (2006) Die Notwendigkeit morphologischer Analysen zur Rekonstruktion der Stammesgeschichte. *Species, Phylogeny and Evolution*, 1, 17–32.
- Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, 56, 564–577.
<http://dx.doi.org/10.1080/10635150701472164>
- Tautz, D. (2006) Morphologie versus DNA-Sequenzen in der Phylogenie-Rekonstruktion. *Species, Phylogeny and Evolution*, 1, 9–16.
- Tan, D.S.H., Ang, Y., Lim, G.S., Ismail, M.R.B. & Meier, R. (2010) From 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera). *Zoologica Scripta*, 39, 51–61.
<http://dx.doi.org/10.1111/j.1463-6409.2009.00408.x>
- Will, K.W., Mishler, B.D. & Wheeler, Q.D. (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology*, 54, 844–851.
<http://dx.doi.org/10.1080/10635150500354878>