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An integrative taxonomic classification and description of two new Cryptorhynchinae from Northwest Africa (Coleoptera: Curculionidae)

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Abstract: Two new species, *Acalles romani* and *Echinodera philippei*, are described from Algeria and Morocco and are distinguished from related species. They are incorporated in the current key to the North African *Acalles* and *Echinodera* species. In addition to morphological characters, first molecular results (DNA barcoding) are presented. The new taxa are included in phylogram of the most closely related species from Northwest Africa, Spain, Italy and the Iberian Peninsula.

Keywords: *Acalles* - *Echinodera* - new species - morphology - molecular analysis - barcoding - integrative taxonomy - distribution map - Morocco - Algeria.

INTRODUCTION

In recent years, research and collecting trips to North African countries have become increasingly difficult for European scientists. It is therefore all the more gratifying that the colleagues Roman Borovec (Czech Republic) and Philippe Ponel (France) recently succeeded in collecting the two new Cryptorhynchinae species herein in Morocco and Algeria. Algeria in particular is still a *terra incognita* for Cryptorhynchine research, particularly in large parts of the Tell Atlas, the wildly rugged Kabylia, the Ouarsensis Mountains and the Sahara Atlas. Similar to what has already happened throughout much of Morocco (Stüben, 2001, 2002, 2003, 2004b), dozens of still undescribed species can be expected to be found here, especially in the genera *Acalles* Schoenherr, 1825*, Kyklioacalles* Stüben, 1999 and *Echinodera* Wollaston, 1863, but also in the tribe of the difficult to detect, subterranean Torneumatini (Stüben *et al.*, 2010; Stüben, 2018).

As already described elsewhere, the range of the *Acalles sierrae* group (Stüben, 2004a), which is widespread on the Iberian Peninsula, continues on the other side of the Strait of Gibraltar into the mountains of Morocco, including the Rif Mountains, the Middle and High Atlas as well as the Anti-Atlas (Schütte & Stüben, 2015: fig. 7). More than a dozen species from this group have become known so far, usually inhabiting a narrowly limited distribution area and often only found on a few mountain peaks (ice age relicts?). So far, the first author has only been able to describe *Acalles testensis* Stüben, 2003 from the Tizi-n-Test and *Acalles asniensis* Stüben, 2003 from the surroundings of Imlil. These were the most eastern species known from the High Atlas, but Roman Borovec has now succeeded in discovering the next species of the *sierrae* group, 150 km northeast of these localities, on Jbel M'Goun (2774 m, Fig. 1). It is expected that, provided safe travel for scientists to such areas is possible, new species will be discovered in each mountain range which is separated from its neighbours by deep, dry valleys. In several ways, the overall 'continental' distribution pattern in many aspects resembles the situation resulting from presumably similar evolutionary process on the Macaronesian islands, where each island lead probably to the evolution of a new sibling species due to the geographic isolation.

A detailed traditional description of the new species, which would only repeatedly record the few, always the same, morphological characters of all *Echinodera* species and the species from the *Acalles* sierrae group, has been omitted this time in favour of more meaningful differential diagnoses. In addition, molecular phylogenies (Fig. 7, 13) are presented for the new and related species and DNA barcoding sequences from all species in the phylogeny trees are deposited in GenBank, the sequences of the new species are being provided within this manuscript (Appendix 1), which will allow a clear (re) identification of the new taxa in the future.

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Fig. 1. Roman Borovec at the 'sunshine selection' of his sifting of the new species *Acalles romani*, named after him, on the Jbel M'Goun of the High Atlas at an altitude of over 2700 m. Numerous other species of the *Acalles sierrae* group can be expected on other such mountain tops of the Middle-, High- and Anti-Atlas (photo by R. Borovec).

MATERIAL AND METHODS

The material of Acalles and Echinodera species on which the morphological and molecular analyses are based was collected, prepared and identified by the first author – except for the two species newly described here, which were provided by P. Ponel and R. Borovec. The morphological reference material is part of the Western Palaearctic Cryptorhynchinae collection of the first author in Senckenberg, Deutsches Entomologisches Institut (SDEI, Germany: Müncheberg). The sequences of the two new species were provided by the laboratories of the SDEI. All the other sequences were provided by the laboratories of the Alexander Koenig Research Museum (ZFMK, Germany: Bonn) in cooperation with the Curculio Institute as part of the Molecular Project for the Identification of Weevils (MWI, Schütte *et al.,* 2013 and Stüben *et al.,* 2015) or previous collaborations (Astrin & Stüben, 2008, 2010; Astrin *et al.,* 2012).

In both facilities the widely used Folmer DNA barcode region (Folmer *et al.*, 1994) of Cytochrome C oxidase subunit I gene (CO1) has been sequenced. The barcodes of the new species were amplified with weevil-adopted LCO1490-JJ and HCO2198-JJ primers (Astrin & Stüben, 2008), obtaining 658 nucleotides per sequence for full length barcode. The sequenced specimens are deposited in the first author's collection and in ZFMK. The extracted DNA samples are stored in ZFMK and SDEI.

Details on the specimens, finding spots and corresponding GenBank accession numbers are provided in type material sections of the new described species and for all other *Acalles* and *Echinodera* species in Astrin & Stüben (2010) and Schütte & Stüben (2015).

The Bayesian analysis of 43 *Acalles* sequences and 36 *Echinodera* sequences was carried out using MrBayes 3.2.7a for Windows (Ronquist & Huelsenbeck, 2003). The nucleotide substitution model HKY+I+G (code = metmt nst = 2 rates = invgamma) was designated, as previously suggested by jModelTest 0.1.1 (Posada, 2008). The Bayesian analysis was run for 20 million generations (samplefreq = 1000). Average standard deviation of last split frequencies for *Acalles*: 0.002323, and for *Echinodera*: 0.003038 (<0.01 suggested). The standard setting of two independent analyses (Nruns = 2) calculated 40,002 trees in 2 files. After absolute burnin of 40 trees per file, 39,922 trees have been retained and a 50%-majority rule consensus tree with posterior probability values was built (Figs 7, 13). Geneious Pro 6.5.8 was used for graphical display of the tree.

TAXONOMY

Acalles romani Stüben spec. nov.

Figs 1-7

Type material: Holotype, 1♂, "MOROCCO, Haut Atlas, M'Goun, Tizi-n-Ait Ahmed, N side, 2774 m, 31°32'57"N 6°16'13"W, 16.V.2017, R. Borovec lgt.", coll. Senckenberg, Deutsches Entomologisches Institut (SDEI Coleoptera #300891). DNA-types (CO1) (= holotype): collector's no.: 3421-PST, GenBank: MW021556. – Paratypes, 3♂, 1♀, data as for holotype, coll. Stüben, Borovec, SDEI.

Description and differential diagnosis: Length: 2.2 - 2.8 mm (without rostrum). The species corresponds in most external characters to the species *Acalles iblanensis* Stüben, 2015, from the Middle Atlas north of Imouzzer-des-Marmoucha on the Jbel Bou Iblane previously described by the first author (in Schütte & Stüben, 2015: see Fig. 4). Unlike this species, however, the long oval and acuminate elytra are not marbled dark and light brown, but are uniformly brown and are 1.3x - 1.4x longer than broad (length-width ratio of the elytra of *A. iblanensis*: 1.2x -1.3x). Similar to the species from the Middle Atlas the clubbed bristles on the sutural stripe and intervals are at most 2.5 to 3 times as long as wide and are separated by two to three times the length of a bristle. The striae are smaller than the intervals, with elongate, closely packed shallow punctures. The laterally strongly rounded pronotum is very similar to *A. iblanensis* (with the exception of the marbled integument), with a slight depression at the sides directly behind the fore-margin. But the most significant differences between the two species are found in both sexes in the shape and length of the red brown rostrum: in males length-width ratio of 3.5 (versus 4.0 in *A. iblanensis*) between insertion of antennae; closely covered with white and beige scales in front of base, finely punctate towards apex; rostrum of females clearly longer; length-width ratio of 4.3 (versus 5.5) between insertion of antennae, more shiny, more finely punctate.

However, a definite distinguishing character is the shape of the median lobe of the aedeagus, which in *A. romani* is always laterally undulating. The different inner structures of the sac of aedeagus are also a great help, see Fig. 5. Figure 6 shows the different female genitalia.

Morphological classification of the new species: The 'Key to the species of *Acalles* Schoenherr, 1825' in Stüben (2018: 44), which contains over 40 species, must be supplemented by the following additions:

[1] Aedeagus with sclerotized internal sac (*Acalles* s.str.) / [2'] External elytral intervals without bump-like elevations / [3'] Elytra elongate-oval, at least 1.25x as long as wide / [30'] Male only with a apically split-tibial spine / [31'] Elytral bristle at least 2.5x as long as wide / [32] Marbled elytra more short-oval: length-width ratio 1.2 to 1.3 ....***Acalles iblanensis* Stüben, 2015**

[32'] Monochrome brown elytra more elongated: length-width ratio 1.3 to 1.4 ....***Acalles romani* spec. nov.**

Fig. 2. Holotype (male) of *Acalles romani* spec. nov. from Morocco (Jbel M'Goun), specimen size = 2.9 mm, scale bar 2 mm.

Fig. 3. Paratype (female) of *Acalles romani* spec. nov. from Morocco (Jbel M'Goun), specimen size = 2.9 mm, scale bar 2 mm.

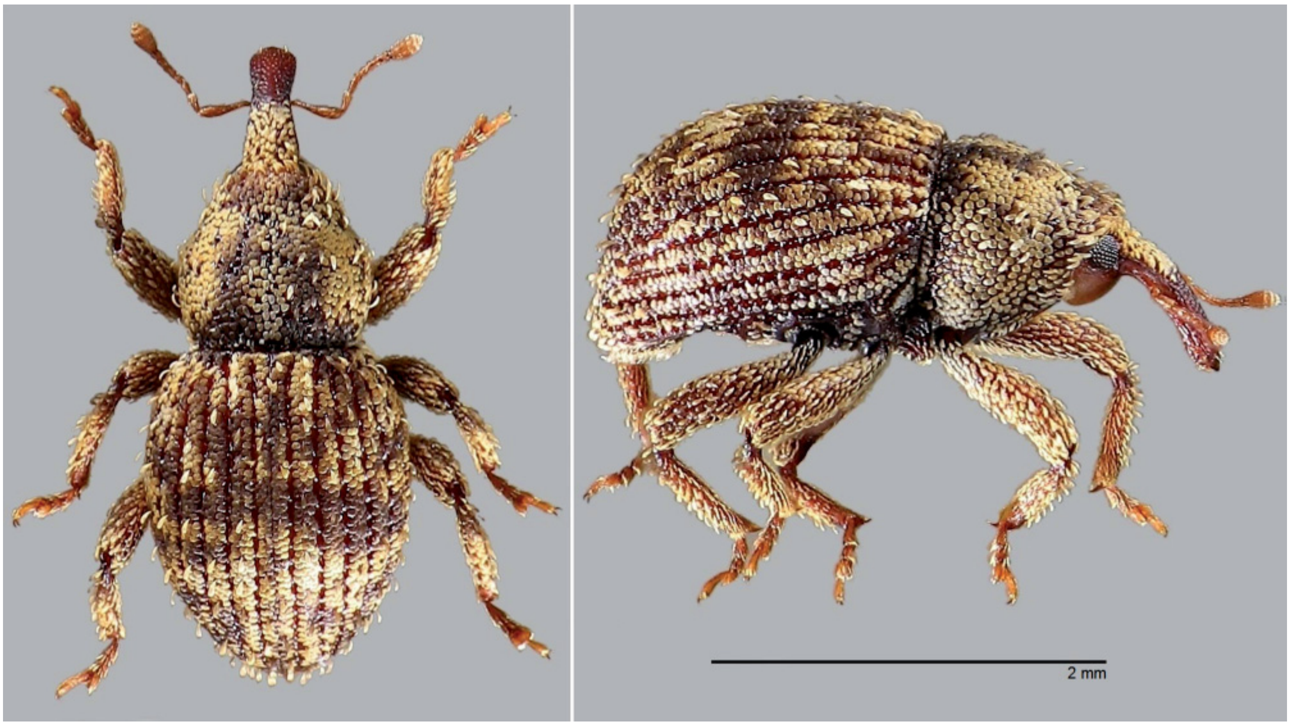

Fig. 4. Holotype of *Acalles iblanensis* Stüben, 2015 from the Middle Atlas of Morocco, specimen size = 2.6 mm, scale bar 2 mm.

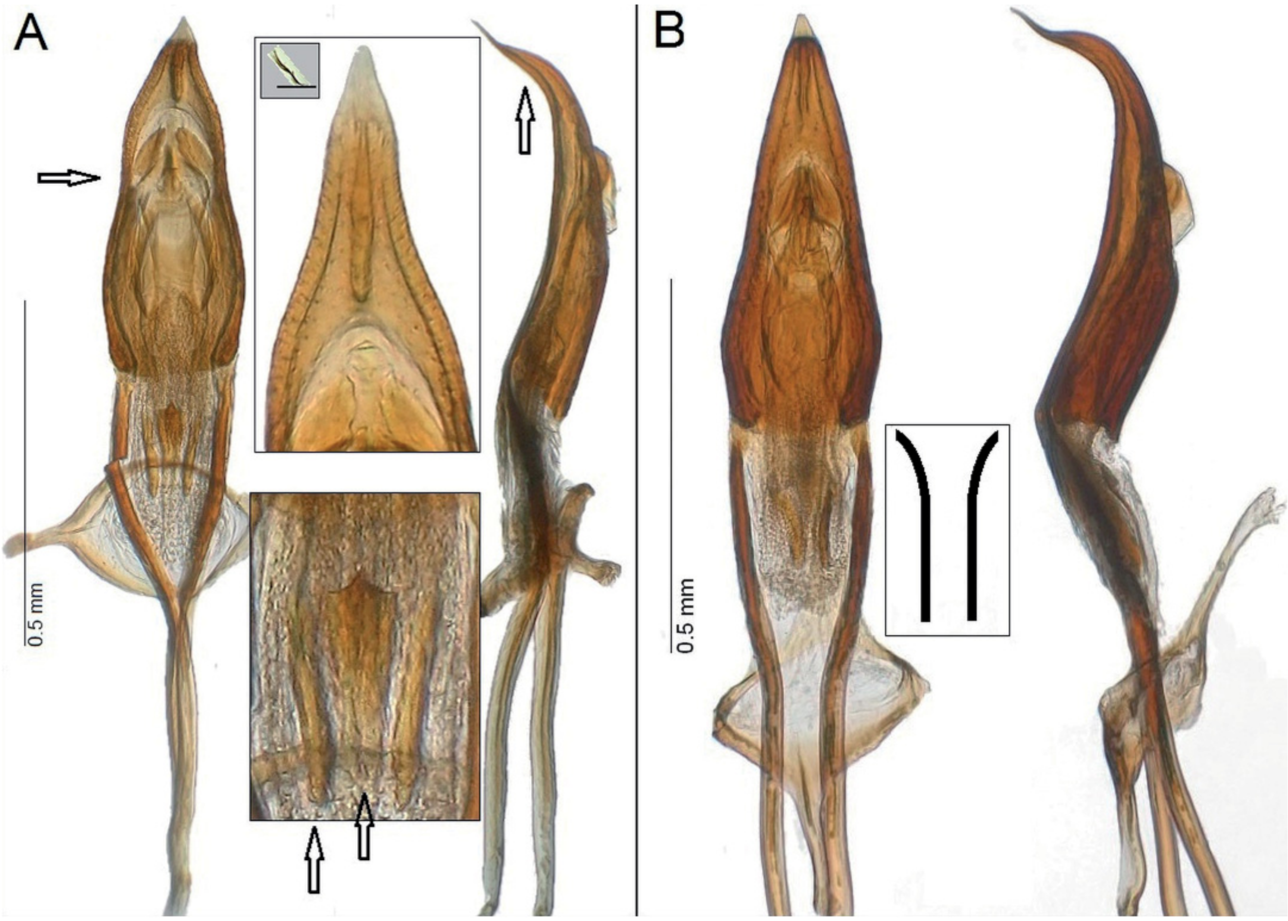

Fig. 5. Aedeagi of (A) *A. romani* spec. nov. and (B) *A. iblanensis*.

The species is thus to be placed in the current key to *Acalles* between *A. iblanensis* from the Middle Atlas and *A. testensis* Stüben, 2003 as well as *A. asniensis* Stüben, 2003 from the High Atlas of Morocco.

Molecular classification of the new species: Methods for obtaining the COI sequence and the process of the Baysian analysis are presented in the section 'Material and methods' (see above). This is not already a more comprehensive or robust phylogenetic classification, which would naturally require additional gene segments, especially nuclear genes (see Astrin *et al.,* 2012; Schütte & Stüben, 2015). Nevertheless, this first molecular classification of the *Acalles sierra* group (see Fig. 7) shows a great concordance with the morphological classification. All these species are highland species from the mountain peaks of the Iberian Peninsula and North-West Africa, almost always above 2000 m a.s.l. The adelphotaxa of the new species are *Acalles testensis* and *A. asnienis* from the western part of the High Atlas, and all together form the sister clade of *A. iblanensis* from the Middle Atlas of Morocco. The uncorrected p-distances in percent of mtCOI subgene between *Acalles romani* sp.n. and *Acalles testensis* / *A. asiensis* are 9% / 7.2%, and the distance between the new species and *A. iblanensis* is 10.2%. Even from this point of view and in comparison with the interspecific molecular distances of the more than 40 species of the genus *Acalles* (cf. Schütte & Stüben, 2015), everything argues for an independent species in case of *A. romani*.

Dedication: I dedicate the species to my esteemed colleague Roman Borovec from the Czech Republic (Kamýcká), who generously made the small series of the new species available to me.

Ecology: *A. romani* was sifted together with *Cathormiocerus jeanneli* Ruter, 1941 (Entiminae) by Roman Borovec under a cushion-like Fabaceae (perhaps *Astragalus*) at an altitude of over 2700 m a.s.l. in the High Atlas of Morocco.

Distribution: This species is so far known only from the type locality (Jbel M'Goun) in the High Atlas of Morocco.

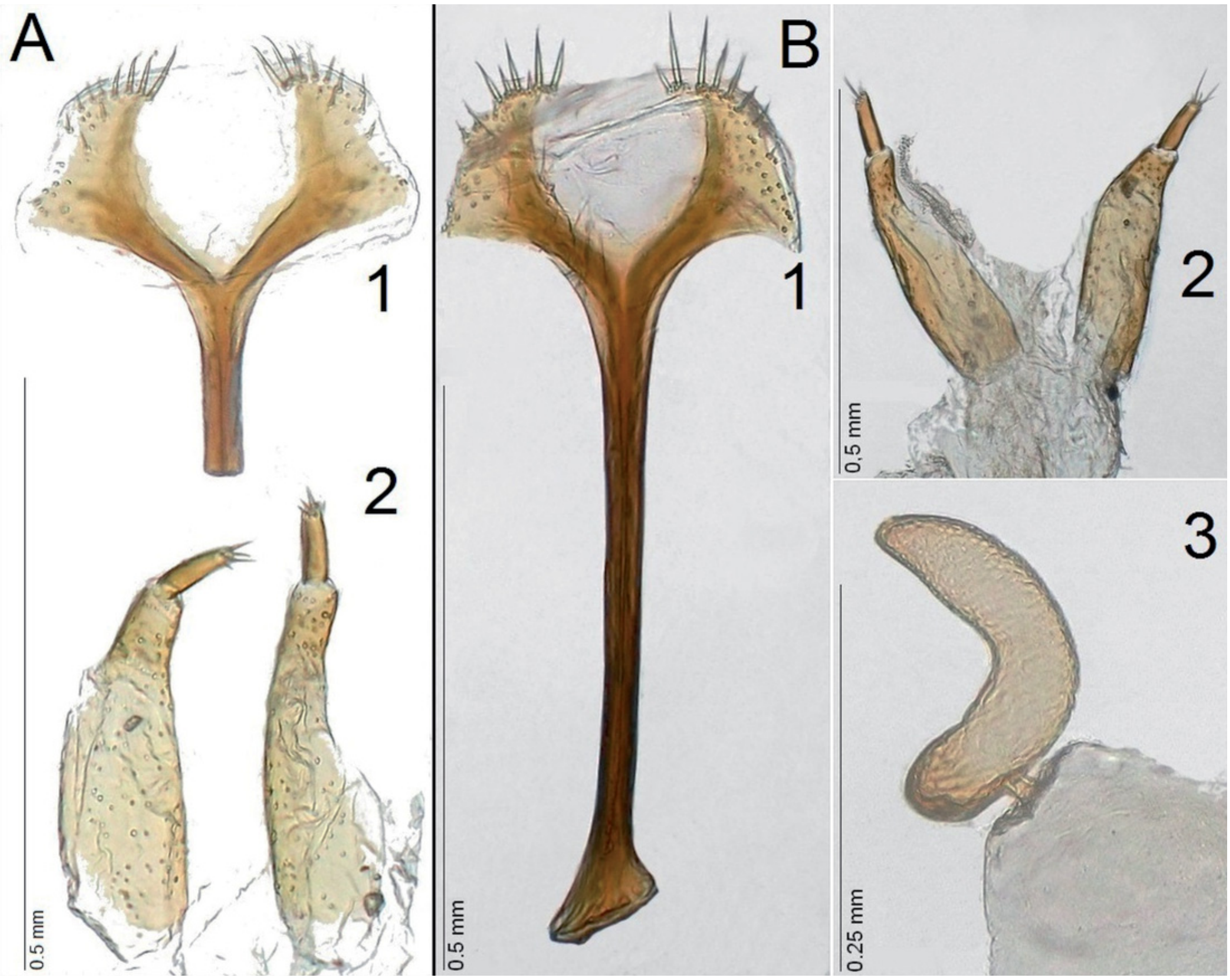

Fig. 6. Female genitalia (1 Spiculum ventrale, 2 Ovipositor, 3 Spermatheca) of (A) *A. romani* spec. nov. (paratype) and (B) *A. iblanensis* (paratype).

Echinodera philippei **Stüben spec. nov.**

Figs 8-11, 13, 14

Type material: Holotype, 1♂, "ALGERIE, Route N22, 6 km de Tlemcen vers Terny Ben Hadiel, litière *Quercus ilex*, 15.1.2018, P. Ponel // N34°46'39" W01°20'46". – Paratypes, 2♂, 3♀, data as for holotype, coll. Stüben, Ponel, SDEI. DNA type (CO1) (= further paratype, data as for holotype): collector's no.: 3190-PST, GenBank: MW021555.

Description

Length: 2.70 - 3.75 mm (without rostrum).

Pronotum: 1.15x - 1.25x as wide as long; widest point behind the middle, in front of the base laterally strongly rounded, towards the fore-margin straight or with a slight depression; disc of pronotum (lateral view) flat, in dorsal view with a large and dense punctures. Every puncture with a short bristle; pronotum mostly covered with dark brown scales, in the center in front of the base with some bright scales as well as bright and irregularly bordered spots on the sides.

Elytra: Short-oval, males 1.04x -1.08x as long as wide (females sometimes more elongated, long-oval); widest at the end of the first third of the elytra, laterally equally rounded; in lateral view, contour-line of the elytra, which slightly surpasses the line of the pronotum, begins with a flat circular line and it drops down vertically towards the apex. The base of elytra is straight. The integument comprises more or less uniformly dark and light brown, irregularly bordered spots of scales. A beige, "M"-shaped fascia, typical for most species of *Echinodera*, is only adumbrated behind the middle on the first three intervals, but can also be completely missing. The striae are clearly slenderer than the slightly arched intervals; these are covered with a single row of bristles, which are raised significantly above the surface (further raised on the elytral slope); the flattened bristles are conically widened (clubbed) towards the tip, 1.5x - 2x as long as wide and

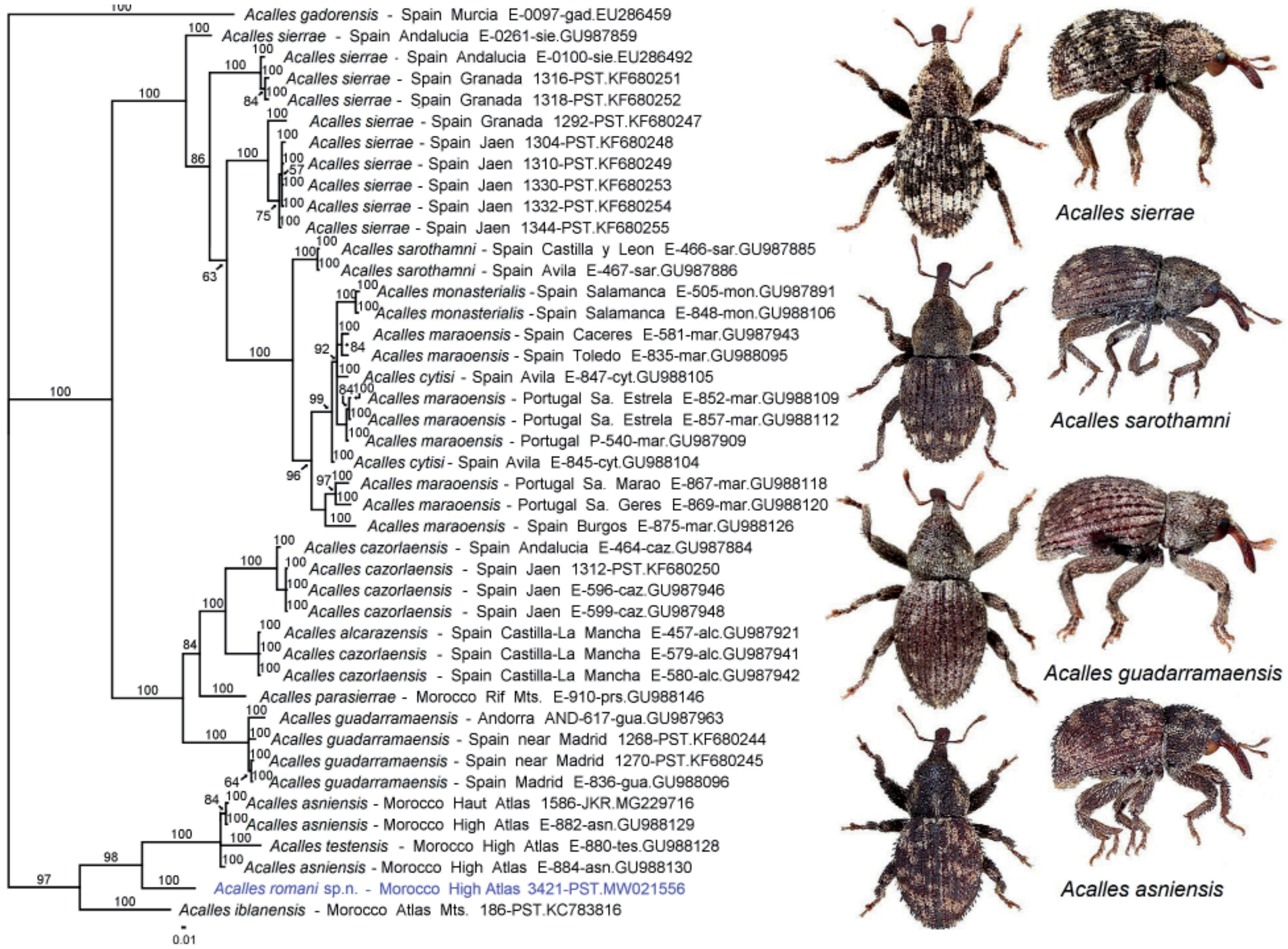

Fig.7. Bayesian 50% majority rule consensus tree using Cytochrome c oxidase subunit I gene (CO1, 658 bp) to classify the new species *A. romani* (marked in blue) among the related species of the *Acalles sierrae* group from Northwest Africa and Iberian Peninsula. Order: Genus / species / location / collector's no. / GenBank acc. no. (CO1).

separated by 0.5 - 1.0x of a bristle-length; 30-35 bristles are placed in a row on the first intervals, they stand more densely packed on the slope of the elytra.

Head & Rostrum: Eyes long-oval, placed at the sides of the head; in dorsal view only visible as narrow stripes and not reaching up beyond the upper margin of the antennal groove; the dark brown rostrum of male deeply and closely punctate and 3.2x as long as wide (breadth of rostrum measured between insertion of antennae); rostrum of female also with deep punctures, slenderer and at least 4x as long as wide.

Legs: Short; front femora reach the fore-margin of the eyes, hind femora reach the fore-margin of the last abdominal segment. Spine at the end of the hind tibia of males curved inwards almost at a right-angle.

Venter: 1st abdominal sternite clearly longer than the three following sternites together.

Aedeagus: Median lobe ventrally not strongly curved, with an S-shaped, long and tapering, transparent and bottleneck-shaped tip (Fig. 10A).

Female genitalia: See Fig. 11A.

Morphological classification of the new species: The 'Key to *Echinodera* from Northern Africa' in Stüben (2018: 318) with more than 80 species must be supplemented by the following additions:

[1'] Spine at the end of the hind tibia of males curved inwards almost at a right-angle (Fig. 11B1) / [7'] Elytral bristles not at least 4x - 6x longer than wide, much shorter (1.5x - 2x as long as wide) / [8'] Elytral striae clearly slenderer than the intervals (not with deep and wide punctures) / [11'] Elytra of males short oval: < 1.08x as long as wide / [16'] Elytral intervals – at least on the elytral slope – with nearly vertically erect bristles (not predominantly recumbent) / [21'] Only the intervals of the elytral slope with vertically erect and shorter bristles (not the entire surface of the elytra with 'hedgehog-like' and longer bristles).

The new species, originating from the northwest of Algeria, must be placed at the very end of the current key to the North African *Echinodera* (Stüben, 2018) and is morphologically similar to *E. hoceimaensis* and *E. ceutaensis* from the North of Morocco, but especially

Fig 8. Holotype (male) of *Echinodera philippei* spec. nov. from Algeria (near Tlemcen), specimen size = 3 mm, scale bar 2 mm.

Fig. 9. Paratype (female) of *Echinodera philippei* spec. nov. from Algeria (near Tlemcen), specimen size = 4.2 mm, scale bar 2 mm.

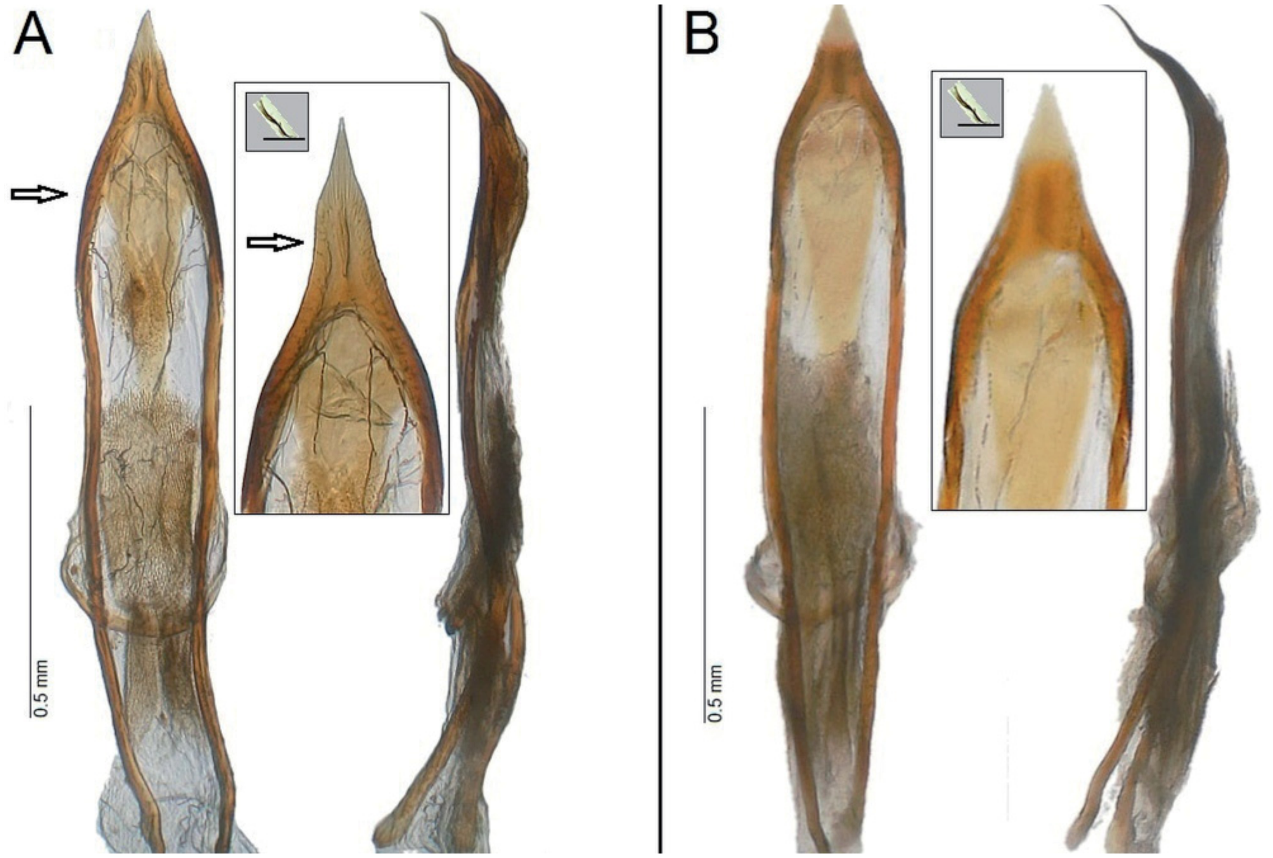

Fig. 10. Aedeagi of (A) *E. philippei* spec. nov. and (B) *E. capbonensis capbonensis* Stüben, 2004b.

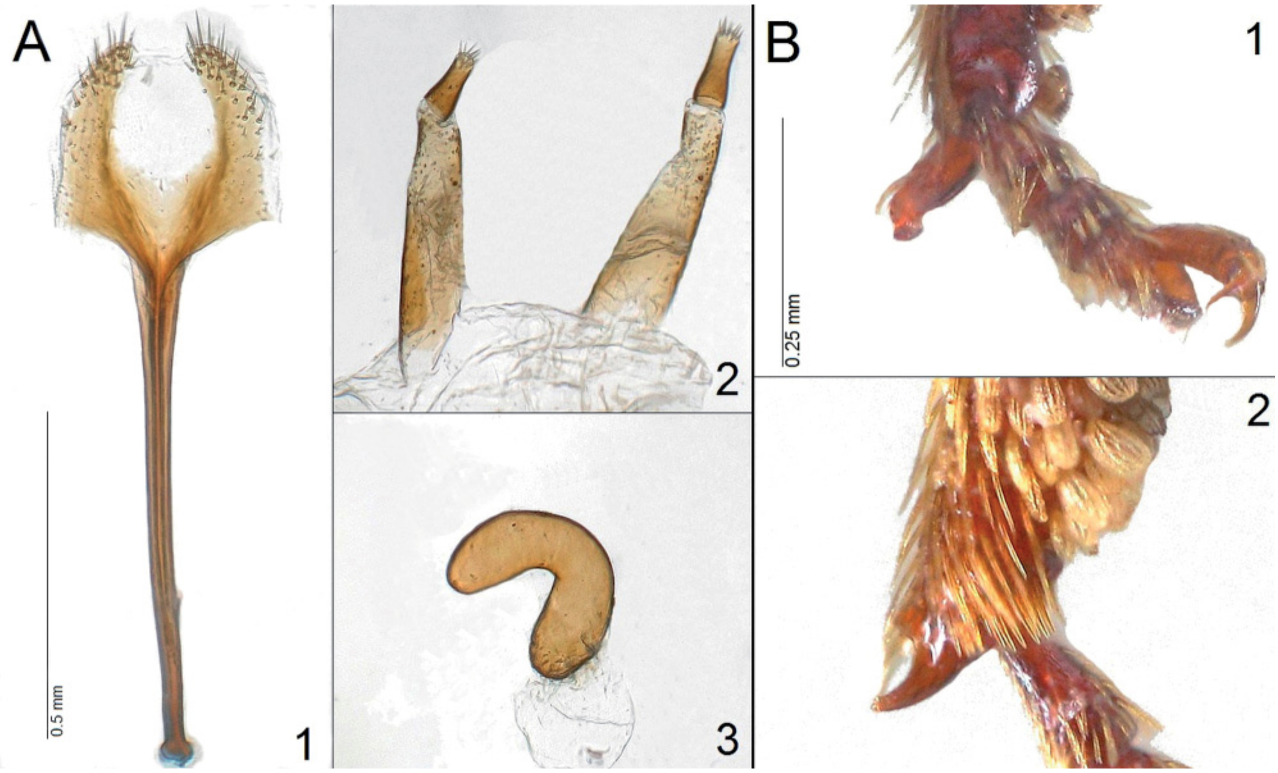

Fig. 11. (A) Female genitalia of *E. philippei* spec. nov. (1. Spiculum ventrale, 2. Ovipositor, 3. Spermatheca). (B) Spine at the end of the hind tibia of males of 1. *E. philippei* spec. nov. and 2. *E. germanni.*

Fig. 12. Holotype (male) of *Echinodera capbonensis diotti* Stüben, 2010 from the island Pantelleria (Italy).

to *Echinodera capbonensis capbonensis* Stüben, 2004 from Tunisia and the further subspecies *Echinodera capbonensis diotti* Stüben, 2010 from the Italian island Pantelleria (Fig. 12). However, *E. philippei* differs from both (sub)species by the narrower and more finely punctate striae and clearly wider bristles on the elytra; in addition, the aedeagus becomes narrower towards the apex and is more tapered like a bottle neck (Fig. 10A). The morphological separation from the poorly-known species *Echinodera fusca* (Chevrolat, 1861) and *Echinodera costata* (Chevrolat, 1861), both described from Algeria, could be problematic. However, the lectotype of *E. fusca* (a female), which I already designated in 2001, has a much more strongly and deeply punctured pronotum and clearly narrower elytral bristles, and the species *E. costata* has much wider and more deeply punctured striae on the elytra (see illustrations of both species in my internet catalogue in: Stüben, 2020). But with only a limited number of taxonomically informative characters available in the very similar ('cryptic') *Echinodera* species (see also the illustrations of some *Echinodera* species in Fig. 13), morphological classification can only serve for the (re)identification of species; e.g. in the form of a species key. Therefore, a classification showing the relationships of species is here only possible using molecular methods, which provides many more 'characters and signals' (see also Astrin & Stüben, 2010).

Molecular classification of the new species: Most of the 82 *Echinodera* species are distributed around the Mediterranean, only a few are found in the Caucasus region, and those of the subgenus *Echinodera* s.str. occur in the Canary Islands and the Salvage Islands. The molecular phylogenetic analysis of all these species using the genes CO1, 16S and 28S can be found in Astrin & Stüben (2010), Astrin *et al.* (2012), and at last almost completely in Stüben, 2018 (pp. 9, 254).

The Bayesian 50% majority rule consensus tree of the mtCO1 gene presented here is based on 36 samples of 10 (of the total of 82) closely related *Echinodera* species from Northwest Africa, the Canary Islands (Fuerteventura), Italy (mainly from Sicily) and the Iberian Peninsula (Fig. 13). In this tree, *Echinodera philippei* sp.n. appears – initially surprising from a morphological point of view – as sister taxon of *Echinodera germanni*, a species described by the first author from the High Atlas of Morocco (Stüben, 2003). However, this species from the Tizi-n-Test, a mountain pass located about 800 km westwards, has somewhat elongated elytra in both sexes (at least 1.23x as long as wide), but is similar to the new species in the length and width of the elytral bristles, their dense placement and in the cloudy coloration of the elytra. But there is a difference that cannot be overlooked: The male of *Echinodera germanni* Stüben, 2003, has a straight spine at the end of the hind tibia and not – like the new species – an inwardly curved, almost right-

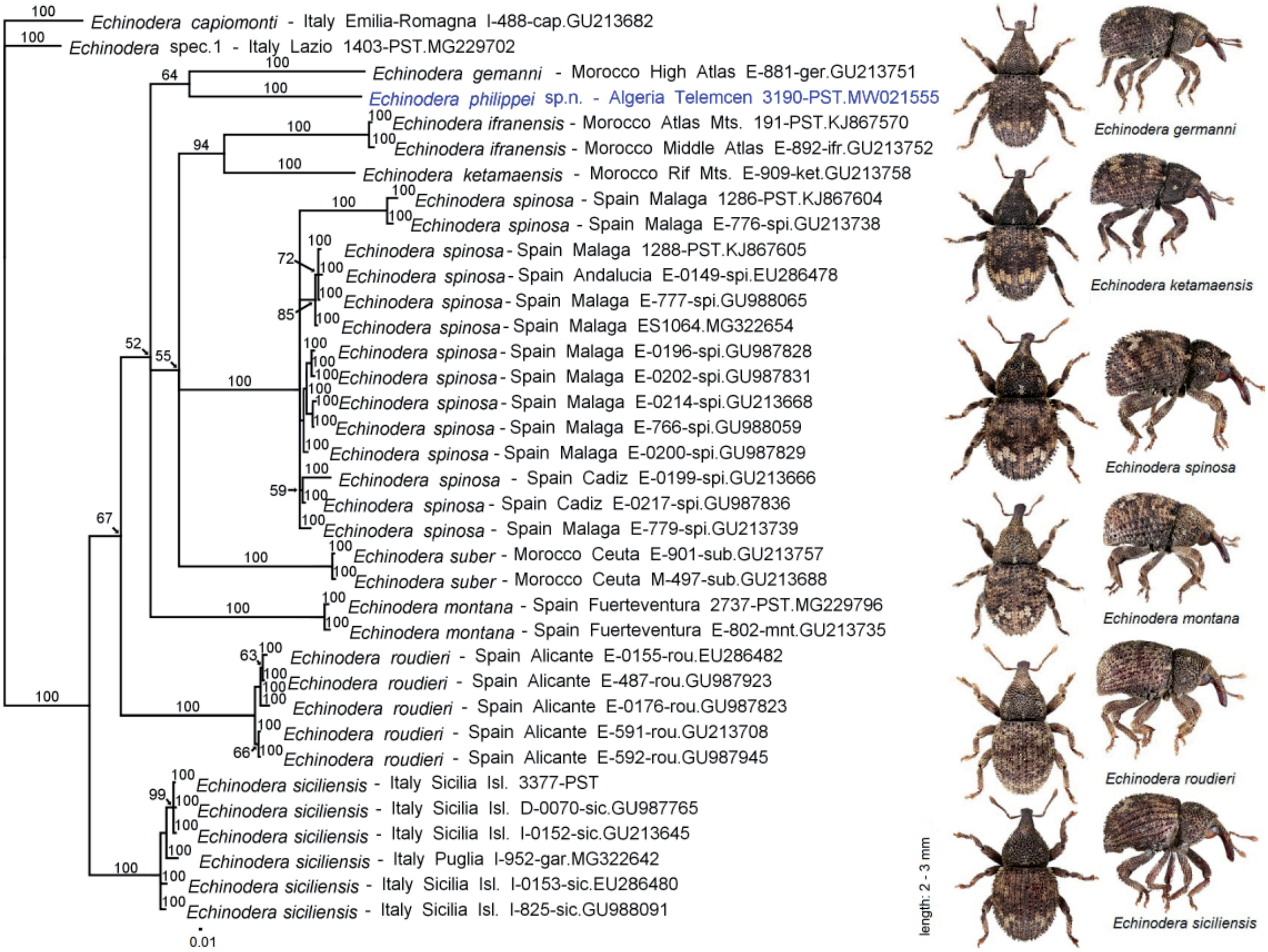

Fig. 13. Bayesian 50% majority rule consensus tree using Cytochrome c oxidase subunit I gene (CO1, 658 bp) to classify the new species *E. philippei* (marked in blue) among the related species of *Echinodera* from Northwest Africa, the Canary Islands (Fuerteventura), Italy and the Iberian Peninsula. Order: Genus / species / location / collector's no. / GenBank acc. no. (CO1).

angled hook (Fig. 11B2). If this molecular placement of the new species as a sister taxon of *E. germanni* is accurate, it shows once again that the former division into the subgenera (*Echinodera* s.str. with a straight spine and *Dieckmania* with an inwardly curved spine) is phylogenetically not tenable, but still very useful for a morphological (re)identification of *Echinodera* species (e.g. in a key, see above or Astrin & Stüben, 2010).

The uncorrected p-distance of the mtCO1 gene between the two sister species from Morocco and Algeria is with 16.7% exceptionally high for *Echinodera* species! This suggests that – with an average distribution radius of *Echinodera* species of about 100 km (cf. Stüben, 2018) – there are probably still many related species to be discovered between these distant sites (assuming safe travel for scientists to such areas is possible).

Dedication: I dedicate the species to my esteemed colleague and long-time member of the Curculio Institute Philippe Ponel from France (Pourcieux), who brought the small series of the new species from Algeria.

Ecology: *Echinodera philippei* was sieved from the litter under *Quercus ilex* by Philippe Ponel at an altitude of over 1100 m a.s.l. near Tlemcen in the north-west of Algeria (Fig. 14). The cold and humid relic forest consists of a mix of *Q. ilex* and Q. *faginea* ssp. *tlemcenensis* (A. DC.) Greuter & Burdet there with a very thick layer of dead leaves (in lit. Ponel).

Distribution: This species is so far known only from the type locality near Tlemcen from the north-west of Algeria near the border with Morocco.

ACKNOWLEDGEMENT

We are grateful to Eva Kleibusch from Senckenberg, Deutsches Entomologisches Institut (SDEI, Müncheberg) for the preparation of the molecular sequences of the

Fig. 14. Type locality near Tlemcen in the north-west of Algeria of the new species *Echinodera philippei*, collected by sieving under *Quercus ilex* (photo by Philippe Ponel / Curculio Institute, bottom right).

new species. Of course, our special thanks go to Roman Borovec (Czech Rep.) and Philippe Ponel (France) for providing the specimen of the new species. We thank Andrew Liston (SDEI) for revising the English text.

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Appendix 1

COI sequences determined for this study

>*Acalles_romani*_spec._nov._3421-PST_GenBank_MW021556
AACTTTATATTTTATCTTTGGGGCATGATCAGGAATATTAGGAACCTCATTAAGTATACTAATCCGAACTGAACTTAGTAACCCAGGAACAATAATTGGAAATGACCAAATTTATAATAGAATTGTTACTGCTCATGCTTTTATCATAATCTTTTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTTCCACTAATACTAGGAGCCCCAGATATAGCCTTTCCCCGATTAAACAATATAAGATTTTGGTTACTCCCCCCATCTTTAACCCTACTATTAATAAGTAGAATTATCGACAAAGGAGCTGGGACTGGATGAACAGTCTATCCCCCACTATCAGCTAATATTGCACATGAGGGAATCTCTGTTGATTTAGCCATTTTTAGCCTCCACATAGCAGGAATCTCATCAATTTTAGGAGCTATCAATTTTGTATCTACAATAGCCAATATACGGCCTGCAGGAATAAATCTAGATAAAATACCCCTATTTTCTTGAGCTGTAAAAATTACAGCTATTCTACTACTCTTATCTCTACCAGTCTTAGCTGGAGCTATTACTATACTCTTAACAGATCGTAATATTAATACATCATTTTTTGATCCTGCTGGAGGAGGAGATCCTATCCTTTACCAACACCTATTT

>*Echinodera_philippei*_spec._nov._ 3190-PST_GenBank_MW021555
AACCCTTTACTTTATCTTCGGATCATGATCCGGAATAGTGGGAACATCTTTAAGTTTACTAATTCGTGTAGAACTAGGAAACCCAGGAACCCTAATTGGAAATGATCAGATTTATAACACAATCGTAACGGCCCATGCCTTTATCATAATTTTTTTTATAGTTATACCCATCATAATTGGAGGCTTCGGTAATTGATTAGTCCCTTTAATGCTAGGCGCCCCTGATATAGCCTTCCCCCGACTAAATAATATAAGATTCTGATTGCTACCCCCATCCTTAACTCTTCTCCTAATAAGAAGAATTATTGATAAAGGAGCTGGGACCGGTTGAACAGTATACCCCCCTTTATCCTCTAATATTGCCCATGAGGGAGCCTCAATTGACTTAGCCATCTTCAGGCTCCACATAGCTGGAATCTCTTCGATTCTCGGAGCTATAAATTTTATCTCAACAGTAATTAATATACGTCCAACAGGTATAAAACTAGATCGAATACCTTTATTCGTTTGAGCCGTAAAAATTACAGCTATTCTTCTCCTCCTATCCCTCCCTGTCCTAGCAGGGGCTATTACCATACTCTTGACCGACCGAAACATTAATACGTCATTTTTCGACCCCGCTGGAGGAGGAGATCCCATCCTTTATCAACACCTTTTC