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The western Palaearctic species of the *Pterostichus nigrita* (Paykull) complex, with the description of a new species from Spain and a new subspecies of *P. nigrita* from Anatolia (Coleoptera, Carabidae)

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Abstract.– The western Palaearctic species of the *Pterostichus nigrita* species complex are revised. Four species, *P. nigrita* (Paykull), *P. rhaeticus* Heer, *P. fuscicornis* (Reiche & Saulcy) and *P. carri* sp. n. (from the Iberian Peninsula and Morocco) are recognised, as well as *P. nigrita pontonigrita* subsp. n. (from Anatolia). *P. pseudorhaeticus* Solodovnikov is placed as a synonym of *P. rhaeticus*. All are recognised by the form of the inflated endophallus. *P. nigrita nigrita* is widely distributed in Europe apart from the Iberian Peninsula, and across Siberia to the Primorye and Kamchatka. It also occurs in the mountains of Kyrgistan, Kazakhstan and Uzbekistan. *P. n. pontonigrita* is known only from Anatolia. *P. rhaeticus* is known from northern and central Europe and extends over Siberia and northern Kazakhstan to the Amur region. *P. fuscicornis* is known from northern Israel, Syria, Lebanon, extreme eastern Anatolia, the Transcaucasus and Iran as well as the eastern Tian Shan in Sinkiang, China. *P. carri* is widely distributed in the Iberian Peninsula, and also occurs in Morocco. Variation in the chaetotaxy of the endophallus is discussed and damage to the bursa copulatrix of females caused the by endophallic chaetae is noted. Karyotypes, with localisation of rDNA by FISH hybridisation, are given for *P. nigrita*, *P. rhaeticus* and *P. carri*. The NOR-bearing autosome of *P. carri* is clearly different from those of *P. nigrita* and *P. rhaeticus*, but all three species may have blocks of rDNA included in the heterochromatin of the B-chromosomes. The chromosomes of *P. fuscicornis* and *P. nigrita pontonigrita* are unknown.

Résumé.– Les espèces ouest-paléarctiques du complexe *Pterostichus nigrita* sont révisées. Quatre espèces, *P. nigrita* (Paykull), *P. rhaeticus* Heer, *P. fuscicornis* (Reiche & Saulcy) et *P. carri* sp. n. (de la Péninsule Ibérique et du Maroc) sont reconnues, ainsi que *P. nigrita pontonigrita* subsp. n. (de l'Anatolie). *P. pseudorhaeticus* Solodovnikov est mis en synonymie de *P. rhaeticus*. Toutes les espèces se reconnaissent par la forme de l'endophallus après inflation. *P. nigrita nigrita* est largement distribué en Europe sauf dans la Péninsule Ibérique, de la Sibérie à la Province Maritime et au Kamchatka. Il se trouve aussi dans les montagnes de Kirghizie, du Kazakhstan et de l'Ouzbékistan. *P. n. pontonigrita* est connu seulement d'Anatolie. *P. rhaeticus* est connu de l'Europe septentrionale et centrale et s'étend de la Sibérie et du Nord du Kazakhstan jusqu'à la région de l'Amour. *P. fuscicornis* est connu du nord d'Israël, Syrie, Liban, partie orientale de l'Anatolie, Transcaucasie ainsi que l'est du Tian Chan, Sinkiang, Chine. *P. carri* est largement distribué dans la Péninsule Ibérique mais aussi au Maroc. La variation de la chétotaxie de l'endophallus est discutée et les dommages causés à la bourse copulatrice des femelles faites par les soies endophalliques sont notés. Les caryotypes avec la localisation de l'ADN recombinant par hybridation fluorescente in situ sont donnés pour *P. nigrita*, *P. rhaeticus* et *P. carri*. L'autosome porteur de la NO-reductase de *P. carri* est nettement différent de ceux de *P. nigrita* et de *P. rhaeticus*, mais les trois espèces peuvent avoir des segments d'ADN recombinant inclus dans l'hétérochromatine des chromosomes surnuméraires. Les chromosomes de *P. fuscicornis* et de *P. nigrita pontonigrita* sont inconnus.

Key-words.– *Pterostichus nigrita*, new species, new subspecies, new synonymy, Carabidae, karyotype.

Introduction

The status of *Pterostichus nigrita* (Paykull) and *P. rhaeticus* Heer as distinct biological species was demonstrated by KOCH & THIELE (1980), as a result of breeding experiments. The importance of the endophallus, right parameres, bursa copulatrix and eighth abdominal sternite in identifying the species was discussed and illustrated by KOCH (1984), who also described the type specimens. The structure of the endophallus of the two species was described with greater precision by ANGUS, BROWN & BRYANT (2000). These authors proposed a nomenclature for the regions of the endophallus, and demonstrated that its chaetotaxy varied in both species. In addition, they demonstrated that the form of the right paramere was an unreliable character for identification of males. They also showed that the apparent differences between the chromosome numbers of the two species resulted from the presence in both species of a variable number of heterochromatic B-chromosomes.

In April 2001 R. B. Angus and J. Galián made a joint collecting trip in Spain, to obtain further material of *P. nigrita* and *P. rhaeticus* for chromosomal and molecular analysis. Study of this material showed that it was referable to neither of these species, but was in fact new. It is here described as *P. carri* sp. n. A second trip, in April 2002, by R. B. Angus, yielded further material.

The discovery of a new species of the *P. nigrita* group in the Iberian peninsula required further research to discover whether material standing as *P. nigrita* from the other Mediterranean projections of southern Europe (Italy and the Balkans) and North Africa might include *P. carri* or any other undescribed species. Investigation of museum material has shown that all south European material so far studied is *P. nigrita*, but that *P. carri* occurs in Morocco. It also confirms that *P. fuscicornis* (Reiche & Saulcy), described from beside the river Jordan, is a fourth species of this complex, widely distributed in the Transcaucasus and Iran, where it replaces other species of the *P. nigrita* group. The only exception to this replacement is provided by specimens from Anatolia which appear to include a distinct form of *P. nigrita* in the dorsal projection (apical lobe) of the endophallus is either reduced or completely absent. This material is considered to represent a distinct subspecies of *P. nigrita*, here described as *P. nigrita pontonigrita* subsp. n.

Study of extensive material of the four species mentioned above leads to the conclusion that the only reliable way of separating these species is by the form of the inflated endophallus, although the form of the right paramere tends to be characteristic of various populations within these species.

Material and methods

The Spanish material collected by Angus and Galián, mentioned in the introduction, has now been augmented by material in the collections of the Zoology Department of the University of Murcia (UM), the Natural History Museum in London (NHML), the Zoological Museum of the Humboldt University in Berlin (MNHUB), the Naturkundemuseum in Erfurt (NME) and the Zoological Institute of the Russian Academy of Sciences in St. Petersburg (ZIN), as well as the private collections of R. Carr of Maidstone, Kent (RC), P. Bulirsch of Prague (PB) E. and P. Hajdaj of Ježov (E&PH) and D. W. Wrase (Berlin) (DW) and G. Chaladze (Tbilisi) (GC).

Preparation of the endophallus

The methods used for preparing the endophalli of fresh material were described by ANGUS *et al.* (2000). In the present work the endophalli of dried or preserved material have also been used. In this case the beetle is first softened in hot water, then the aedeagus is removed and placed for 6 – 24 hours in 5% sodium hydroxide at room temperature. The shorter times are for specimens which are found to be partially decomposed. The aedeagus is then transferred to distilled water and any adhering soft tissue is removed. The endophallus is inflated as for fresh material, with first water then absolute alcohol being injected. This is not always successful, but often inflation is achieved. In some cases the endophallus may be overinflated or distorted, especially if the gonopore is blocked with fragments of soft tissue. In some cases there is partial collapse associated with critical-point drying, but this is often minimal, and some very good preparations have been obtained.

An alternative approach, adopted by G. Chaladze and developed from a method published by O. BERLOV (1992) involves inflating the endophallus with toothpaste and then drying it. Dried material may be softened in a mixture comprising 75 ml 95% ethanol, 20 ml distilled water and 5 ml glacial acetic acid. The aedeagus is fastened to the end of an adapted insulin syringe (outer diameter of needle about 0.31 mm, inner diameter about 0.15 mm) which has been filed down to eliminate the "chisel-tapered" end, and also to reduce its diameter further to allow insertion into the basal hole of the aedeagus. The toothpaste should be of a soft-creamy texture (not a gel). Some of the preparations obtained by this method are excellent (Fig. 3 E – G, L, M), but the very extended basal lobes may be artefact. The scanning electron micrograph (SEM) pictures of endophalli in the present study were taken with unplated material, using back-scattered electrons. They are almost as good as the images obtained by using secondary electron emissions from gold-plated material (ANGUS *et al.*, 2000), and leave the endophallus in a natural state for future examination. The photographs of the toothpaste-inflated preparations show the dark setae very clearly.

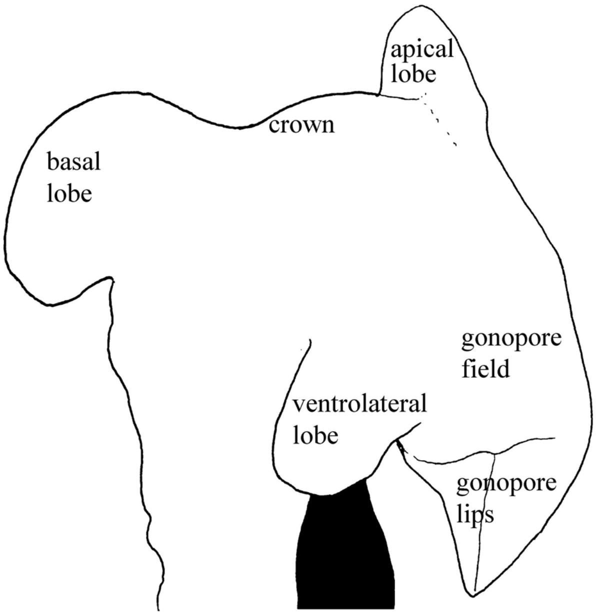

Fig. 1.– Inflated endophallus of *Pterostichus nigrita*, left side view, to show the parts and structures used in the descriptions. Adapted from ANGUS *et al.*, 2000.

The measurements used to point up the differences between the right parameres of *P. nigrita* and *P. rhaeticus* (ANGUS *et al.*, 2000) are no longer helpful when all four species are considered, and are not used here. Methods for preparing karyotypes and mounting preparations of the bursa copulatrix are described by ANGUS *et al.* (2000).

Chromosome preparations

The methods of chromosome preparation and C-banding were as described by ANGUS *et al.* (2000). In addition, some slides were examined dry, unstained, using low power phase-contrast, and selected slides were sent to J. Galian (Murcia, Spain) for in situ fluorescence hybridisation (FISH) to locate nucleolus organisers and other regions with rDNA, as follows: - The ribosomal probe was obtained as described in DE LA RÚA *et al.* (1996) from *Cicindela maroccana* DNA. FISH was performed as previously described (PROENÇA & GALIÁN, 2003). Briefly, chromosome spreads were pre-treated with DNase-free RNase in 2x SSC for 1 h at 37ºC, followed by treatment with 0.005% pepsin in 10 mM HCl for 10 min. After digestion the chromosomes were fixed with fresh paraformaldehyde in NaOH 0.1 N, dehydrated in a graded ethanol series and air-dried. The hybridisation mixture containing 50% deionised formamide, 2x SSC, 50 mM sodium phosphate (pH = 7.0), 10% dextran sulphate and 4 ng/µl of labelled probe was denatured by boiling for 3 min and placed on ice. The slides were heated on an 80ºC hot plate for 5 min. A 30 µl aliquot of the denatured hybridisation mixture was placed over the denatured slides and covered with a 22 x 22 mm coverslip. The slides were then transferred to a humid chamber at 80ºC, allowing temperature to drop slowly to 37ºC for hybridisation overnight. After hybridisation coverslips were carefully removed and the slides were then given a stringent wash for 3x 5 min in 50% formamide, 2x SSC at 37ºC. Sites of probe hybridisation were detected with avidin-fluorescein isothiocyanate (FITC). The signal was amplified twice using goat anti-avidin-biotin. Slides were counterstained with propidium iodide and mounted with antifade solution to prevent the fluorescence fading away. Slides were examined with a Leitz photomicroscope and photographed with Imation colour film 100 ASA.

The slides were then sent back to R. B. Angus for further processing: they were placed vertically in racks in absolute ethanol to remove the coverslips. They were then refixed overnight in 3:1 ethanol acetic acid fixative, dried and treated for C-banding. After staining and drying the slides were scanned under the microscope and where possible nuclei photographed after FISH treatment were located and rephotographed, to enable comparison of C-banded and FISH treated preparations of the same nucleus.

Structure and chaetotaxy of the endophallus

The structure of the endophallus was figured by ANGUS *et al.* (2000), and a slightly modified version of this illustration is shown as Fig. 1. As noted by ANGUS *et al.* the arrangement of the lobes and projections of the endophallus is characteristic of the various species. All have a distinct basal lobe and a ventrolateral lobe on the left hand side of the endophallus, basal to the gonopore field. Typical *P. nigrita* has a prominent apical lobe rising from the right hand side of the crown, and in *P. rhaeticus* there is a much weaker bulge towards the left side of the crown. In *P. rhaeticus* and *P. carri* there is a slight bulge on the right hand side of the gonopore field, and in *P. fuscicornis* this bulge is very distinct. ANGUS *et al.* (2000) suggested that this bulge may be homologous

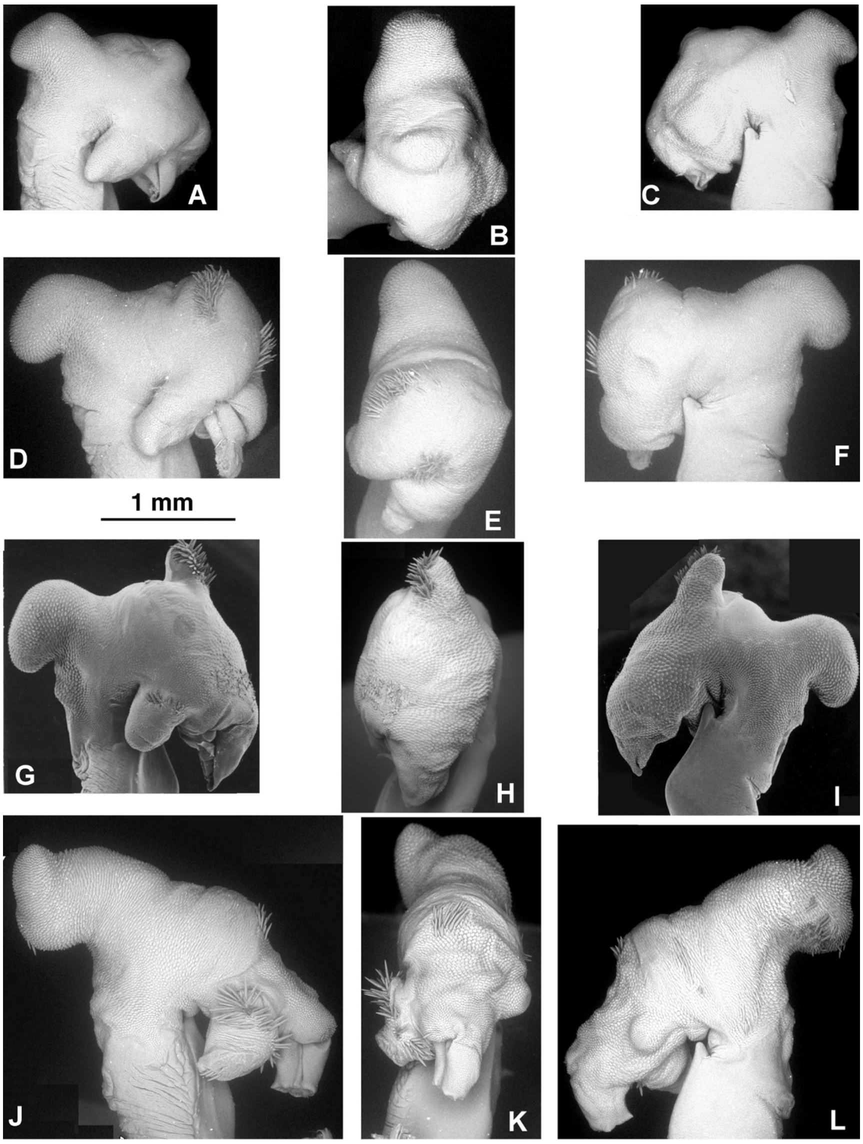

Fig. 2.– SEM photographs of inflated endophalli of *Pterostichus* spp. : A-C.– *P. rhaeticus*, Matley Bog, New Forest, Hampshire, England, fresh specimen. A: left, B: dorsal, C: right. D-F.– *P. carri* sp. n., holotype, Balneario de Corconte, Burgos, Spain, fresh specimen. D: left, E: dorsal, F: right. G-I.– *P. nigrita*, Cothill, Berkshire, England, fresh specimen. G: left, H: anterodorsal, I: right. J-L, *P. fuscicornis*, Tanur river, Upper Galilee, Israel, dried resoftened specimen. J: left, K: anterodorsal, L: right.

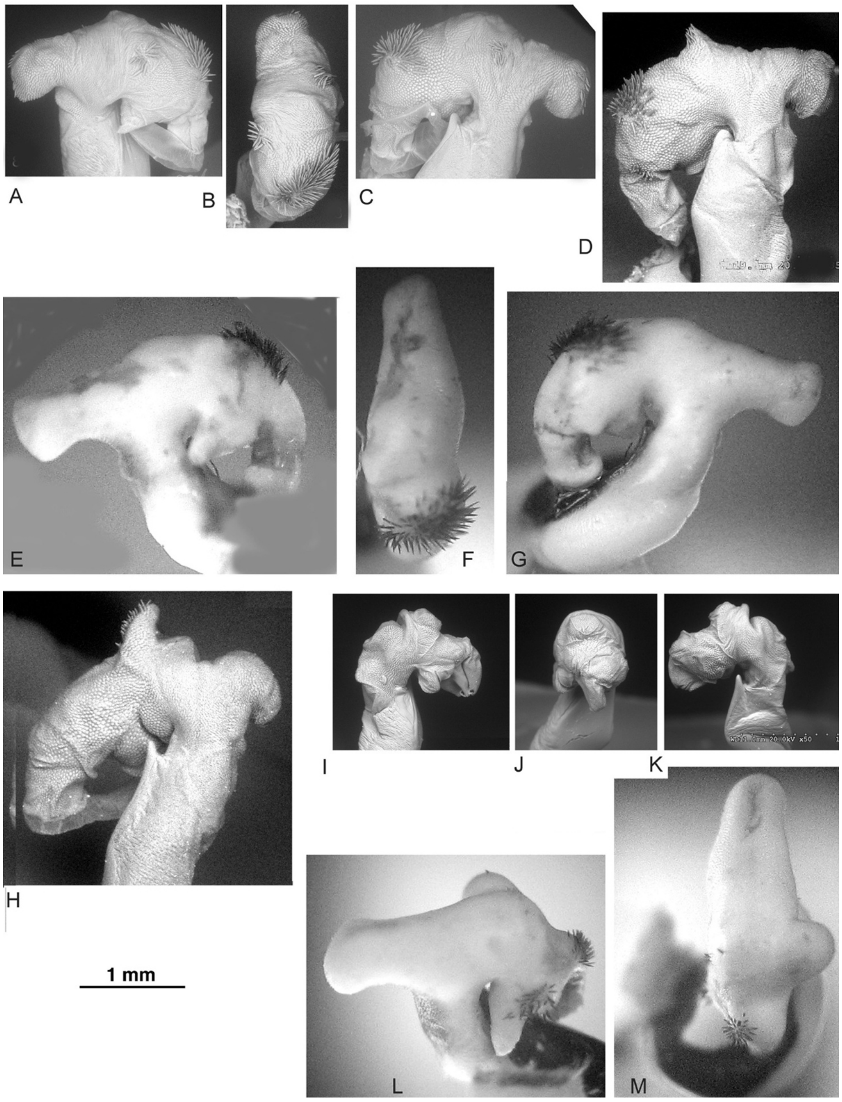

Fig. 3.– inflated endophalli of *Pterostichus* spp. : A-C.– *P. nigrita pontonigrita*, ssp. n. holotype, Ordu, Fatsa, Turkey, dried, resoftened specimen. A: left, B: dorsal, C: right. D.– *P. nigrita pontonigrita*, paratype showing a reduced apical process, Zara, Turkey, dried resoftened specimen, right. E-G.– *P. nigrita pontonigrita*, photograph of toothpaste-inflated paratype, Demirkapi, Rize, Turkey. E: left, F: dorsal, G: right. H.– *P. nigrita* var *rufifemoratus* Stephens, holotype, right. I-K.– *P. pseudorhaeticus* Solodovnikov, holotype. I: left, J: anterodorsal, K: right. L-M.– *P. fuscicornis*, Nadibani, Georgia, toothpaste-inflated specimen. L: left-dorsal, M: dorsal.

with the apical lobe of *P. nigrita*. The endophallus of *P. rhaeticus* is distinctly smaller than those of the other species, so that the distance between the apex of the gonopore lips and a point just below the basal lobe is about 1.35 mm as against about 1.5 mm (compare Fig. 2A and 2 D and G), but the varying degrees of inflation and subsequent partial collapse of the endophalli make accurate measurements impossible.

One of the surprising features of the endophallus of the *P. nigrita* species group is the extreme variability of the chaetotaxy. Angus *et al.* found no evidence of a regional component to this variation, and subsequent work has not altered this impression, though it has shown the chaetotaxy to be even more variable, extending to the basal lobe in some specimens of *P. nigrita*. It is noteworthy that *P. nigrita pontonigrita*, on the basis of material seen to date, always has an oblique field of chaetae across the top of central part of the gonopore field. This field may be present in other species. An indication of the probable role of these chaeta-fields came from the discovery of often extensive injuries to the bursa copulatrix of some females. These individuals showed black or very dark brown areas of healing – pieces of thickened, darkened cuticle which were not affected by the hydrolysis by sodium hydroxide used to clear the flesh from the structures (Fig. 5, E, H, I). These areas are not just pigmented areas, but are discrete, often closely spaced areas of thickened hard dark cuticle, of varying sizes, which can be probed with a dissecting needle – matching the arrangement of endophallic chaetae. This suggests that these chaetae may be designed to injure the female during mating, to render her less likely to have subsequent matings, either because of the pain involved, or the provocation of an immune response. This has already been noted and demonstrated in *Callosobruchus* species (Bruchidae) (CRUDGINGTON & SIVA-JOTHY, 2000).

The *Pterostichus nigrita* species group

The *Pterostichus nigrita* group of species comprises a subsection of the subgenus *Pseudomaseus* Chaudoir, formerly placed in *Melanius* Bonelli. It is here defined as comprising species with the following suite of characters: pronotal sides rounded to distinct slightly protruding hind angles; elytral microsculpture consisting of more or less isodiametric reticulation; last fixed abdominal sternum of males with a distinct tubercle in mid line – not a longitudinal keel; right paramere of males more or less hatchet-shaped, with a narrow basal portion and an expanded apical one, this with a sharp angle at the base of its ventral margin. This is more or less the definition adopted by SOLODOVNIKOV (2001), but the requirement for a discrete tubercle on the last abdominal sternum of males should exclude the Japanese *P. basipunctatus* Straneo, whose genitalia are unknown. The requirement for distinct, slightly protruding, hind angles of the pronotum should, on the basis of Solodovnikov's key, exclude *P. ambigenus* Bates, also from Japan, but this distinction is not clear from examining Bates' type, in the Natural History Museum, London, so that species is considered to be a member of the *P. nigrita* group. SOLODOVNIKOV (2001) describes a number of species and subspecies from the eastern Palaearctic, and these require further investigation, and especially figures of the inflated endophallus, before their statuses can be evaluated. For this reason the present study considers only those species whose distributions extend into the western Palaearctic. This gives four species, *P. nigrita* (Paykull), *P. rhaeticus* Heer, *P. fuscicornis* (Reiche & Saulcy) and *P. carri* sp. n. The chromosomes of all except *P. fuscicornis* are known and their karyotypes are unique among those known from the suborder Adephaga in having a suite of sometimes quite large heterochromatic B-chromosomes.

Pterostichus carri **sp. n.**

HOLOTYPE ♂: Spain, Provincia de Burgos, Balneario de Corconte, 20/21-IV-2002, R. B. & E. M. Angus. NHML. **Paratypes**. From Spain: 2 ♂♂, 1 ♀, data as holotype. 1 ♀, Provincia de Burgos, Arnedo, 20-IV-2002, R. B. & E. M. Angus. 4 ♂♂, 1 ♀, Provincia de Burgos, Balneario de Corconte, 26-IV-2001, R. B. Angus & J. Galián. 1 ♂, Provincia de Palencia, Areños, 26-IV-2001, R. B. Angus & J. Galián. 1 ♂ 1 ♀, Provincia de Segovia, La Salceda, 25-IV-2001, R. B. Angus & J. Galian. These paratypes are divided between the collections of the Museum Nacional de Ciencias Naturales, Madrid, the Naturhistorisches Museum, Wien, R. B. Angus and J. Galián.

Others paratypes. 2 ♂♂, Provincia de Santander (Cantabria), Puerto de Palombera, 11-VI-1990, R. Carr. RC. 1 ♂, 1 ♀, Provincia de Albacete, 11 KM N of El Bonillo, 11-V-2002, J. Galián. UM. 1 ♂, Provincia de Albacete, Rio Guadalimar, 11-V-2002, J. Galián. UM. 1 ♂, 1 ♀, Provincia de Albacete, Laguna de Salobralejo, 18-VI-1982, Dto Biologia leg. UM. 1 ♀, Provincia de Albacete, Riopar, 19-III-1988, J. Lencina leg. UM. 2 ♀♀, Provincia de Soria, Laguna Negra, 10-VII-1991. D. B. An. Murcia. UM. 3 ♂♂, “Pto de Pajares Spain, G.C.C.” (Provincia de Oviedo/Provincia de León). NHML. 1 ♂, “Bejar Spain, G.C.C.” (Provincia de Salamanca). NHML. 1 ♂, “La Estrella Sierra Nevada 8000 ft. G.C.C.” NHML. 4 ♂♂, Provincia de Madrid, Sierra Guadarrama, Rascafria, 1000m, 19-VI-1991, D. Wrase. 3 MNHUB and 1 DW. 1 ♂, Provincia de Granada, Alpujarra, Capileira, 1900 m, 22-III-1994. DW. 1 ♂, Provincia de Jaca, Selva de Ofa, Pirenei Aragonesi, Bord. leg. 20-VII-1984. DW. 1 ♂, Provincia de León, Montes de la Cabrera, Lago de Baña, 1600 m, 10-VI-1996, D. Wrase. DW. 1 ♂, Provincia de León, Puerto de San Glorio, 25-VI-1986, F. Bajet leg. DW. 3 ♂♂, Provincia de Córdoba, PN Sierra de Hornachuelos: Cortijo Cabalgadenos, Aroyo Guadalora, 150 m, 24-II-1999, D. Wrase. 2 NME and 1 DW. 1 ♂, Provincia de Tarragona, Hapsanes, 1-V-1980, J. & E. Vives leg. DW. From Morocco: 1 ♂, Ifrane, Rif Mts, 11-V-1995, P. Bulirsch leg. DW.

All the paratypes have well-inflated endophalli which have minimal collapse on drying.

No material is known from outside the Iberian Peninsula and Morocco, and no specimens morphologically intermediate between *P. carri* and either *P. nigrita* or *P. rhaeticus* have been encountered.

Length (from the anterior margin of the closed mandibles to the elytral apex): 9.3 – 10.5 mm (♂), 9.2 – 12.5 mm (♀). Black. Pronotum with sides evenly rounded to denticulate hind angles.

Basal foveae of pronotum broad, double, heavily punctured. Elytra with three dorsal pores, the epipleurs crossed subapically and with interstices 9 and 10 about as wide as each other, though 9 tends to be slightly wider than 10. Male with segments 1 – 3 of anterior tarsi strongly dilated, segments 1 and 2 about as wide apically as long, and abdominal sternite 7 with small, slightly elongate, median tubercle.

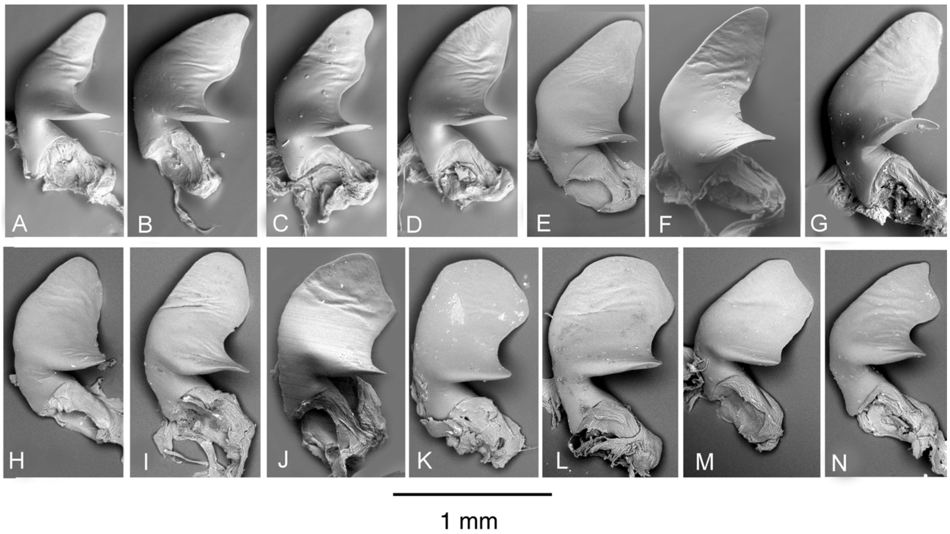

Fig. 4.– SEM photographs of right parameres of *Pterostichus* spp. A.– *P. carri*, holotype. B-D.– *P. carri*, paratypes, Balneario de Corconte, Burgos, Spain. E.– *P. carri*, paratype, Salobreja, Portugal. F-G.– *P. nigrita*, Uzbekistan. H.– *P. nigrita pontonigrita*, holotype. I.– *P. nigrita pontonigrita,* paratype, Cangal Dag, Turkey. J-N.– *P. fuscicornis*. J: Tanur river, Upper Galilee, Israel; K: Akbes, NW Syria; L: Lorestan, Iran; M: Bludan NW of Damascus, SW Syria; N: Zchneti, Tbilisi, Georgia.

Genitalia characters. ♂. Endophallus (Fig. 2 D – F) lacking any upwardly directed apical projection and with upper surface almost smoothly rounded from the crown to the start of the gonopore lips. Size about as in *P. nigrita*, distinctly larger than that of *P. rhaeticus* (Fig. 2 A – C, 3 I - K). Tufts of dark setae variably disposed, but may be present on the basal lobe, the crown, the gonopore field and the ventrolateral lobe. The general shape and size of the endophallus is similar to that of *P. nigrita* (Fig. 2 G – I, 3 H), though more laterally compressed and without any trace of the apical lobe. The endophallus of *P. rhaeticus* is smaller and the anterior part is ridged above the gonopore field (the smaller apical lobe and lateral lobes, as described by Angus et al. (2000)). Right paramere (Fig. 4 A - E) variable, often but not always, narrower than that of *P. nigrita* (Fig. 4 F – I).

♀. Bursa copulatrix (Fig. 5 C - E) with sclerotised area oval, obliquely set, as in *P. rhaeticus* (Fig. 6 F, G), but larger, size either as in *P. nigrita* (Fig. 5 A, B), or larger still, though without the lateral folds of *P. nigrita*. Length of sclerotised section of spermathecal duct varying from almost as long as apical segment of gonocoxae to distinctly shorter. Eighth abdominal tergite (Fig. 5 L - N), with the size and shape more like *P. nigrita* (Fig. 5 J, K) than *P. rhaeticus* (Fig. 5 O, P).

Etymology. Named after Mr Ron Carr of Maidstone, Kent, who first drew my attention to problems with the chromosomes of the *Pterostichus nigrita* group of species.

P. nigrita (Paykull)

Carabus nigrita Paykull, 1790. Lectotype ♀: Koch, 1984

Pterostichus nigrita karnoldii Solodovnikov, 2001. **Syn. n.**

Poecilus rufifemoratus Stephens, 1828. Lectotype ♂, this paper. *P. nigrita* var. *rufifemoratus* Stephens 1828.

P. nigrita pontonigrita **ssp. n.**

The typical form of this species (*P. nigrita nigrita*) is immediately distinguished from all others by the prominent upwardly directed apical lobe of the inflated endophallus (Fig 2 G – I, 3 H) (ANGUS *et al*., 2000). ANGUS *et al*. noted the variation in the chaetotaxy of the endophallus, and this variation is widespread but without any apparent geographical pattern. A selection of right parameres were figured by Angus et al., who showed that they gave only a partial separation of *P. nigrita* and *P. rhaeticus* Heer.

The eighth abdominal sternite and the bursa copulatrix of females were shown by Angus *et al*. to give a clear distinction between *P. nigrita* and *P. rhaeticus*. However, the distinctions are less clear when *P. carri* sp. n. and *P. fuscicornis* (Reiche & Saulcy) are considered. (Fig. 5 J - R).

The type material of Paykull's *Carabus nigrita* was described by KOCH (1984) as comprising two females, one of which she designated Lectotype. She figured the eighth abdominal sternum of the lectotype, leaving no doubt of its identity.

STEPHENS (1828) mentioned that he had never taken his *Poecilus rufifemoratus* near London but he had been informed by Halliday that it was abundant near Belfast. He added that he had recently obtained one specimen from Scotland and two from the north of England, and that one of these two was "of a rich blue above". KOCH (1984) concluded that the type material was in Halliday's collection, but it could not be found. In fact, Stephens did not mention seeing material from Halliday. Stephens' collection (NHML) contains only one specimen, a black red-legged male *P. nigrita*, standing as *P. rufifemoratus*. This specimen is here designated Lectotype of *Poecilus rufifemoratus*. The endophallus (Fig. 3 H) confirms that this is a form of *P. nigrita*. Stephens' other material cannot be found, but his mention of a specimen with a rich blue dorsal surface suggests that more than one species may have been involved.

SOLODOVNIKOV (2001) described specimens of *P. nigrita* from the mountains of Middle Asia (the former Soviet Central Asia) as differing from typical material in having a slightly more inwardly curved basal ventral angle of the expanded apical section of the right paramere, and in the male usually having a pair of setigerous punctures on its anal sternum. In his key he refers to figures of the pronotum which may suggest that the material from Middle Asia has a slightly less transverse pronotum than typical material. Solodnikov described this Middle Asian material as *P. nigrita karnoldii* ssp.

n., but his figure of the pronotum (Fig. 1, 1) is labelled as *P. nigrita kirgisiensis* ssp. n. However, although a locality is given for this specimen, there is no statement about a type or types, so this is not a valid description of a new subspecies (Article 16.4 of the current International Code of Zoological nomenclature).

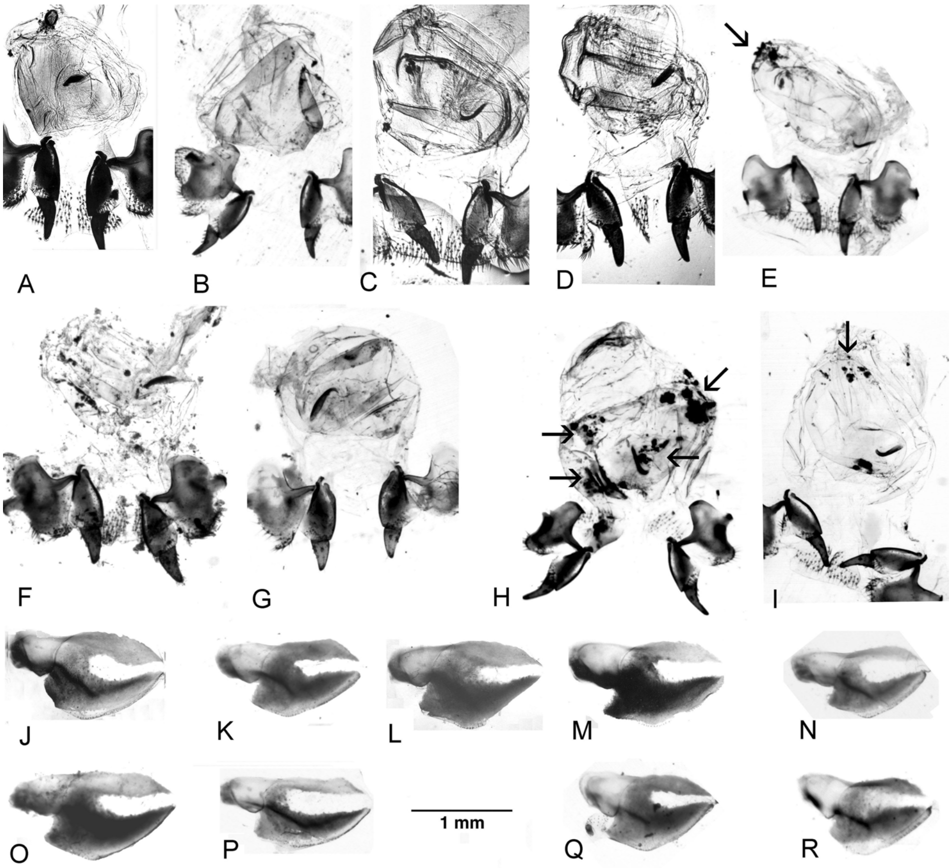

Fig. 5.– Micrographs of the bursa copulatrix and associated structures (A-I) and abdominal sternite 8 (J-R) of female *Pterostichus* species. A, J: *P. nigrita*, Catfield Fen, Norfolk, England; B, K: *P. nigrita pontonigrita*, “Anatolia Bor.”, Turkey; C, L: *P. carri*, paratype, La Salceda, Segovia, Spain; D, M: *P. carri*, paratype, Laguna Negra, Soria, Spain; E, N: *P. carri*, paratype showing damage to bursa (arrowed), caused by spines of male endophallus, Rascafria, Madrid, Spain; F, O: *P. rhaeticus*, Matley Bog, New Forest, Hampshire, England; G, P: *P. rhaeticus*, Krasnoyarsk, W. Siberia, Russia; H, Q: *P. fuscicornis* showing extensive damage to bursa (arrowed), caused by spines of male endophallus, Golan, Israel; I, R: *P. fuscicornis* showing more limited damage to bursa (arrowed), caused by spines of male endophallus.

We have not studied type material of *P. nigrita karnoldii*, but have dissected and examined five specimens from Kazakhstan, Kyrgistan and Uzbekistan. The form of the endophallus is identical with that of normal *P. nigrita*, and the paramere differences appear slight and inconsistent. Fig. 4 F, G shows right parameres of two males from the Uzbek Tian Shan (DW). The ventral angle at the base of the apical section is folded inwards (towards the apex) in one specimen (Fig. 4 G), but not the other (Fig. 4 F). We therefore see no justification for recognising material from Central Asia as representing a separate subspecies.

The typical form of this species, *P. nigrita nigrita*, is very widely distributed in Europe, excluding the Iberian Peninsula. In the south it is known from Italy (including Calabria), Bulgaria, Greece and Turkey and the North Caucasus (the former Kabardino Balkar ASSR) and extends into Georgia as far as Batumi. Further north it is known from Ireland, and extends to Ukhta (Komi republic of Russia), and extends over West and East Siberia to the Primorye and Kamchatka. As already mentioned, it occurs in the mountains of Middle Asia. Material from western Anatolia belongs to the nominate subspecies, but further east it becomes *P. n. pontonigrita* (see below).

Pterostichus nigrita pontonigrita subsp. n.

HOLOTYPE ♂, *Pterostichus nigrita pontonigrita*: TR, Ordu, Fatsa, 50 m, 20-VII-1993. DW. (Eventually to go to MNHUB). Paratypes (12 ♂♂): 2, TR. Muğla, No. 14, SE Köyceğiz, 10 m, flood-plain wood 36°56'50" N, 28°43'56" E. 28-III-2002, P. Wunderle. DW. 1, Turkey m. 24/25-VI-1992, Ala Daglari Demirkazik, ca 1550 m, P. Bulirsch lgt. PB. 1, NC Turkey. 20 km N Zara 1800 m. 40°01' N, 37°43' E. 17-VI-2002, leg. E. & P. Hajdaj. E&PH. 1, Anatolia bor., Heinz leg., Çangal dağ südl. Ayancik. 1000–1300 m. 28-VII-1978. coll. Heinz, Schwanfeld; 1, TR bor or. Şavşat, 22-V-1992, Z. Kinousek. DW. 1, TR, Ordu, 28-V-1989, S. Gölköy (18), leg. Schödl. DW. 1, N. Turkey, Giresun, 5 km SE Kumbet, Şehitler Gec., 2260 m, 40°23'N 38°37'E, 26-VI-2005, lgt E. & P. Hajdaj. E&PH. 1, Turkey, Giresun, ca 35 km S Giresun, 1350 m, 40°35’N 38°26’44”E, (Spruce forest, Rhodod.), 20-VII-2006, M. Schülke (19). DW. 1, Pulümür bei Erzinçan, leg. H. Korge, 18-VI-1964. MNHUB. 1, NE Turkey, Trabzon env., 20 km S Maçka, 1780 m, 44°44'N 39°41'E, E. & P. Hajdaj. DW. 1, Turkey NE, prov. Gumushane, Karakaban Dagi Mts, 1900 m, Vaudagi Gecidi, 21-VI-1998, Pavel Vonička lgt. DW. 2, Turkey, Demirkapi (Rize province). G. Chaladze.

The Ordu, Fatsa specimen is chosen as holotype because its endophallus (Fig. 3 A – C) has scarcely collapsed on drying and closely resembles the toothpaste-inflated specimen from Demirkapi (Fig. 3 E – G) whose endophallus has not collapsed at all on drying. These specimens represent the extreme form of *P. nigrita pontonigrita*, but the holotype still shows a hint of the apical projection.

This subspecies, so far known with certainty only from Anatolia, is characterised by the loss or reduction of the upwardly directed apical lobe of the endophallus (Fig. 3 A - G). Western Anatolian material (Muğla) has the lobe distinct but slightly weaker than in the nominate subspecies. Material from central Anatolia (Zara, Fig. 3 D) and Ala Dağları has the lobe distinct but very weak, while in material from northern (Çangal dağ, Ordu) and northeastern Anatolia (Şavşat) the lobe is either completely absent or almost so, with only a slight darkening of the surface of the endophallus to mark its position. The right parameres of this material (Fig. 4 H, I) are all within the normal range shown by *P. n. nigrita*.

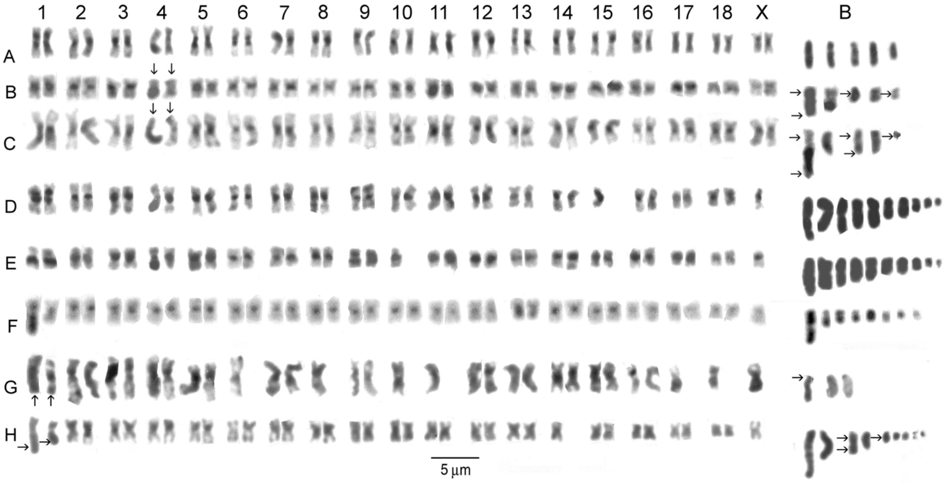

Fig. 6.– Mitotic chromosomes from mid gut of *Pterostichus* spp. shown as karyotypes. A-C.– *P. carri*, paratype ♀, Arnedo, B and C with the position of fluorescent rDNA blocks indicated by arrows: B shown with fluorescent staining in Fig. 7 A and C-banded as Fig. 7 H, C shown fluorescent stained in Fig. 7B and C-banded in Fig. 7 I; D, E.– *P. carri*, holotype ♂, Balneario de Corconte, 1 autosome missing from each preparation; F.– *P. nigrita*, ♂, Cothill, Berkshire, England, shown as Fig. 8 E by ANGUS *et al*., 2000; G.– *P. nigrita*, ♂, Virginia Water, incomplete karyotype with the position of fluorescent rDNA blocks indicated by arrows, shown with fluorescent staining in Fig. 7 C and C-banded in Fig. 7 J; H.– *P. rhaeticus*, ♂, Ockham Common, Surrey, with 1 replicate of autosome 14 missing and the position of rDNA blocks indicated by arrows, shown with fluorescent staining in Fig 7G and C-banded in Fig. 7 N.

P. rhaeticus **Heer**

Pterostichus rhaeticus Heer, 1837. Lectotype ♂: Koch, 1984.
Pterostichus pseudorhaeticus Solodovnikov, 2001. **Syn. n.**

The type material of *P. rhaeticus* Heer was described by KOCH (1984) as comprising one male, which she designated as lectotype, and two females, paralectotypes. She figured the right paramere of the lectotype and described its endophallus, leaving no doubt of the identity of this species.

P. pseudorhaeticus was described by Solodovnikov on the basis of 13 specimens from the Baikal and Amur regions of East Siberia, and we have the Holotype, from Pashkovskoe near Irkutsk, and three male paratypes on loan from ZIN. The endophallus of the Holotype is shown in Fig. 3 I - K. This shows no difference from normal *P. rhaeticus*, and neither does that of a paratype from Bunbuy. Solodovnikov states that his main reason for regarding *P. pseudorhaeticus* as a distinct species is the large gap between its distributional range and that of *P. rhaeticus*, as well as small but constant differences in the form of the right paramere. The paramere of *P. rhaeticus* is variable in form, and Solodovnikov's Fig. 1.4 (*pseudorhaeticus*) shows no obvious difference from the *rhaeticus* figured by him as Fig. 1.8, and the type material at present on loan resembles the *P. rhaeticus* figured by ANGUS *et al.* (2000) as Fig. 4 j, l and m. There is thus no morphological reason for regarding *P. pseudorhaeticus* as a species separate from *P. rhaeticus*. In addition to this, the geographical separation of the European and Siberian material is not so great as Solodovnikov thought, and may well be largely the result of a shortage of material from West Siberia. Thus we now have material at the eastern edge of the "European" range of the species from Pechora (Komi region, 65° 49' N, 52° 36' E) and Tyumen (western Siberia, 57° 0' N, 65° 18' E) and at the western edge of the "East Siberian" range, from Krasnoyarsk (E. Siberia, 56° 8' N, 93° 0' E) and Karkaralinsk (Kazakhstan, 49° 25' N, 75° 28' E).

ANDERSON (2002) recorded a single male *P. rhaeticus* with red femora "the *rufifemoratus* trait" from near Frymore Lough, Northern Ireland. We have this specimen on loan from Dr Anderson and have inflated the endophallus, confirming that it is indeed *P. rhaeticus*. It is interesting to find that in Ireland, the only region where red-legged *P. nigrita* is known to occur in any abundance, red-legged *P. rhaeticus* also occurs.

P. rhaeticus is widely distributed over northern and central Europe, but absent from the south. In Russia it is known from the town of Pechora (Komi republic), Tyumen and Krasnoyarsk in West Siberia, Karkaralinsk in northern Kazakhstan, and from the Baikal and Amur regions of East Siberia. It is unknown in the Caucasus and Middle Asia (apart from Karkaralinsk).

P. fuscicornis (Reiche & Saulcy)

Feronia (*Omaseus*) *fuscicornis* Reiche & Saulcy, 1855

Feronia confusa Chaudoir, 1842, not *Feronia confusa* Dejean, 1831.

This species was known by the name of *P. confusus* (Chaudoir) until CSIKI (1930) showed that this (*Feronia confusa* Chaudoir) is a junior homonym of *Feronia confusa* Dejean, described from near Buenas Aires in Argentina. WRASE (1992) redescribed the holotype of *P. fuscicornis*, a male from the banks of the river Jordan. He figured the aedeagus (a cleared preparation mounted in Euparal) and the right paramere. The general appearance of the specimen, and its right paramere, match material from northern Israel

(Jordan valley, leg. Wrase), whose right paramere and endophallus are shown in Fig 2 J – L and 4 J and which is used as a reference specimen for this species.

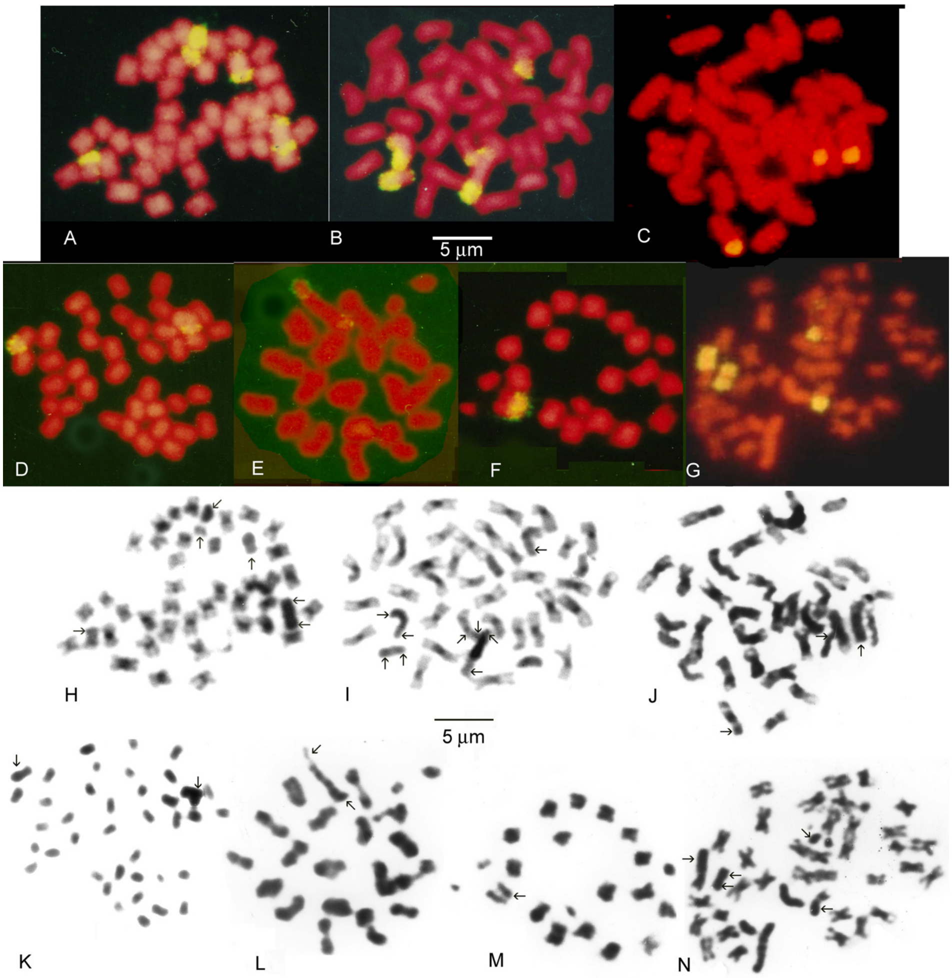

Fig. 7.– Chromosomes of *Pterostichus* spp shown with fluorescent staining following FISH treatment for rDNA (A-G) and afterwards C-banded with the positions of the rDNA blocks indicated by arrows (H-N). A, H: *P. carri*, paratype ♀, Arnedo, karyotype shown in Fig. 6 B; B, I: a second nucleus from the same specimen as A and H, karyotype shown in Fig. 6 C; C, J: *P. nigrita*, ♂, Virginia Water, karyotype shown in Fig. 6 G; D-F, K-M, *P. nigrita*, Virginia Water, testis, D, K: spermatogonial mitosis, E, L: meiosis, metaphase 1, F, M: meiosis, metaphase 2; G, N: *P. rhaeticus*, ♂, Ockham Common, karyotype shown in Fig. 6 H.

The endophallus of *P. fuscicornis* is characterised by a strong lateral subapical protuberance on its right-hand side, and an abrupt drop of its dorsal surface just before the apex. The lateral protuberance is rather small in Israeli and Syrian material, but much stronger in specimens from the Transcaucasus (Fig. 3 L, M). The right paramere has its apical angle rounded in the typical form (Fig. 4 J), but much sharper in Transcaucasian material (Fig. 4 N), which can resemble *P. rhaeticus*. The forms of paramere appear to intergrade. Iranian material includes both forms, with specimens from Lorestan (Fig. 4 L) resembling the typical (Israeli) form in both the small endophallic protuberance and the rounded paramere. Syrian material includes specimens with very rounded parameres (Fig. 4 K) as well as material with a distinct apical angle (Fig. 4 M).

P. fuscicornis is widely distributed in the Transcaucasus and northern Iran, and extends down through Syria and Lebanon to northern Israel. The Zoological Museum of the Humboldt University in Berlin has two males from "East Turkestan" (Sinkiang), labelled "Kok-Teke Geb. 6.1902. coll. Hauser." The Kok-Teke mountains are the Horo Shan, a spur projecting ESE from the eastern Tian Shan at approximately 42^{o} 07' N, 83^{o} 46'. This record suggests an eastwards extension of the range of *P. fuscicornis*, south of that of *P. nigrita*.

Chromosomes

Representative karyotypes of *P. carri*, *P. nigrita* and *P. rhaeticus* are shown in Fig. 6, while FISH stained nuclei are shown in Fig. 7 A – G, with the same nuclei shown C-banded in Fig. 7 H – N.

P. nigrita. Fig. 7 F shows a C-banded karyotype from a male taken at Cothill, Berkshire, England (originally published as Fig. 7 E by Angus et al., 2000), while Fig. 6 G shows a partial karyotype from a male from Virginia Water with the positions of the rDNA indicated by arrows. A FISH stained photograph of this nucleus is shown in Fig 7 C while Fig. 7 J. This specimen shows three rDNA blocks, one on each replicate of autosome 1 and one on one of the B-chromosomes. Fig 7 D – F shows FISH stained preparations of testis from a second Virginia Water male, with the same nuclei are shown C-banded in Fig 7 K – M, with the positions of the rDNA indicated by arrows. In these preparations the C-banding was unsuccessful, and the chromosomes are uniformly stained by the Giemsa. This specimen has only two rDNA blocks, at the distal ends of the long arms of the two replicates of autosome 1. Spermatogonial mitosis (Fig. 7 D, K) shows the blocks at the end of the longest autosome, first metaphase one of meiosis (Fig. 7 E, L) shows the blocks at each end of a large bivalent, while metaphase 2 shows one rDNA-bearing chromosome in a haploid nucleus. These results confirm the suggestion by ANGUS *et al.* (2000) that the longest autosome in this species, sometimes displaying a secondary constriction, is the site of the nucleolus organiser. It is not clear whether the different sizes of the two replicates of autosome 1 shown in Fig 6 G reflect different quantities of heterochromatin, or just differing expansion of the secondary constriction. The two replicates of autosome 1 are clearly larger than the other autosomes in the preparation shown in Fig. 7 D, K. The occurrence of rDNA on some of the B-chromosomes is variable, indicating the heterogeneous nature of their heterochromatin. So far three rDNA blocks per nucleus is the highest number found in *P. nigrita*.

P. rhaeticus. Fig. H shows a C-banded karyotype of a male from Ockham Common, Surrey, with the positions of the blocks of rDNA indicated by arrows. Fig. 7 G, N shows this preparation after FISH staining and subsequent C-banding. One replicate of a small autosome is missing. As in *P. nigrita*, autosome 1 is shown to be the site of the nucleolus organiser, and also to vary in length between the two replicates. In this specimen there are a further three rDNA blocks on the B-chromosomes, two on a fairly large B-chromosome and one on a small one. As in *P. nigrita*, the number of B-chromosomes bearing rDNA varies, and three is the highest number found so far. The C-banding has worked well on this preparation.

P. carri. C-banded karyotypes from a female paratype from Arnedo, Burgos, Spain are shown in Fig. C A – C and from the holotype male from Balneario de Corconte, Burgos, Spain, are shown in Fig. 6 D, E. These two preparations each lack one autosome (a different one in the two preparations), but allow identification of the X chromosome as one of the smaller ones in the karyotype. The positions of the rDNA blocks are indicated by arrows in Fig. 6 B, C, and FISH stained preparations of these nuclei are shown in Fig. 7 and after subsequent C-banding in fig. 7 H, I. As in the other two species the heterochromatin of the B-chromosomes includes blocks of rDNA, with the largest B-chromosome showing two distinct blocks, and the third largest showing either two blocks (one very small, terminal) or just one larger block, depending on its degree of condensation. More significant taxonomically is the position of the rDNA (nucleolus organiser) on a pair of autosomes. Unlike the other two species, the NOR-bearing autosome is not obviously the largest, and it is placed as pair no. 4 in the karyotypes shown in Fig. 6. Further, this autosome has a clearly heterochromatic long arm (in one replicate at least, so possibly heterozygous) which is not the site of the main rDNA (NOR), and one replicate (Fig 6 C, 7 B, I) shows a very small terminal rDNA block at the distal end of this heterochromatic long arm.

Discussion

The four species of the *P. nigrita – rhaeticus* complex investigated here show great similarity in their appearance, including karyotypes, and it is fortunate that additional data confirming the status as separate species have been found for *P. nigrita*, *P. rhaeticus* and *P. carri*. Thus Koch and THIELE (1980) reported that laboratory reared populations of *P. nigrita* and *P. rhaeticus* failed to hybridise, even after attempted copulation, under conditions in which both species bred successfully. It may be noted that the karyotypes of these species show no detectable inter-species differences in either their autosomes or X chromosomes. No such experimental data are available for *P. carri* but the karyotype of this species is clearly distinct in the relative length of the NOR-bearing autosome, implying translocation of chromosomal material when compared with *P. nigrita* and *P. rhaeticus*, and hence reproductive incompatibility with these species. No chromosomal data are available for *P. nigrita pontonigrita* and *P. fuscicornis*. The geographical distribution of *P. n. pontonigrita* and the existence of forms intermediate between it and *P. n. nigrita* leave no room for doubt that these are conspecific, but different subspecies. *P. fuscicornis* appears a very clearly distinct taxon, with aedeagal differences comparable with those between the other species of the complex, so there is no reason to doubt its status as a distinct species. The variation in the shape of the right paramere and the strength of the lateral protuberance of the endophallus has already been noted, and it may be that subspecies are involved here, but for the moment the geographical basis does not seem clear enough to describe subspecies.

The overall similarity between these species is reflected by data on their molecular phylogeny. Preliminary data from an 810 base-pair fragment of the mitochondrial gene CO1 show extreme similarity between *P. nigrita*, *P. rhaeticus* and *P. carri*, indicating a very recent divergence of the species. One male each of *P. nigrita* and *P. rhaeticus* from Ockham Common, Surrey, and seven *P. carri* from three localities in northern and central Spain have been sampled. No nucleotide change within *P. carri* was detected, finding a single haplotype. The only variation found is in position 168, where the *P. rhaeticus* has A, while the *P. nigrita* and all the *P. carri* have G and position 333, where the *P. nigrita* has T, the *P. rhaeticus* has A and all the *P. carri* have G. This level of differentiation between haplotypes is extremely low when compared with intra or interspecific diversity in other Carabidae, such as the tribe Harpalini (MARTINEZ-NAVARRO *et al.*, 2005), ranging from around 1% between populations and 5 % between species.

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