Mantophasmatodea: A New Insect Order with Extant Members in the Afrotropics

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A new insect order, Mantophasmatodea, is described on the basis of museum specimens of a new genus with two species: *Mantophasma zephyra* gen. et sp. nov. (one female from Namibia) and *M. subsolana* sp. nov. (one male from Tanzania). This is the first time since 1914 that a newly described extant insect taxon has proved unplaceable within a recognized order. Mantophasmatodeans are apterous carnivores. Their closest phylogenetic relationships may be to Grylloblattodea (ice-crawlers) and/or Phasmatodea (stick insects), but the morphological evidence is ambiguous. *Raptophasma* Zompro from Baltic amber is assigned to the Mantophasmatodea, revealing a wider previous range for the lineage.

All of the currently recognized orders of extant insects have been known throughout most of the 20th century. While several high-rank clades of marine animals have been discovered during the past 50 years, the numerous new insects described in that period have all been referable to known orders [with the possible exception of the apterygote *Tricholepidion gertschi* Wygodzinsky, 1961, whose assignment to the Zygentoma remains disputable (*1–3*)]. The recognized insect order based on the most recently discovered extant taxon is the Grylloblattodea (= Notoptera, "ice-crawlers"), the first of whose 26 currently known species was described in 1914. We here report the finding (*4*) of a new Afrotropical insect genus that cannot be placed within any recognized extant order, and whose phylogenetic relationships to extant or extinct orders remain unclarified. A new order is therefore established to accommodate these insects as well as *Raptophasma kerneggeri* Zompro, 2001, from Baltic amber, hitherto classified as "Orthoptera incertae sedis."

Classification (*5*): Order Mantophasmatodea Zompro, Klass, Kristensen et Adis, ord. nov. Family Mantophasmatidae Zompro, Klass, Kristensen et Adis, fam. nov. Type genus: *Mantophasma* Zompro, Klass, Kristensen et Adis, gen. nov., with two species (extant), known from one ethanol-preserved female specimen (Fig. 1, B and C) and one dried, shriveled, teneral adult male specimen (Fig. 1A), respectively. Other included genus: *Raptophasma* Zompro, 2001, with one species (from Baltic amber).

Etymology: *Mantis* was the Linnaean name for mantises (including stick insects), and *Phasma* is a classical generic name for stick insects; the name alludes to the superficial similarity of all the insects in question.

Order and family description (*6*): Development of the external genitalia indicates that both available specimens of *Mantophasma* are adults. Head hypognathous (Fig. 1, D to F) with generalized mouthparts. Mandibles only with three small teeth in molar area. Palps five-segmented (maxillae) or three-segmented (labium). Antennae long, filiform, multisegmented. Ocelli absent. Tentorium without perforation, anterior pit far above anterior mandibular articulation. Epistomal sulcus lacking. Subgenal sulcus with unusual course, extending from posterior mandibular articulation directly to anterior tentorial pit and thence downward/backward to anterior mandibular articulation (Fig. 1E). Head capsule posteroventrally closed by weak submentum (no gula).

Thorax with each tergum narrowly overlapping the following. Prothoracic pleuron large, fully exposed (Fig. 1F). Wings entirely lacking. Metathorax without spinal apodeme. Coxae elongate. Tarsi (Fig. 1, G and H) with five tarsomeres, four basal with euplantulae. Three basal tarsomeres synscleritous, borders distinctly indicated by grooves. Dorsal membrane beyond third tarsomere with triangular process (Fig. 1I). Pretarsal arolium very large, with row of long setae (Fig. 1J).

In abdomen, tergum I and coxosternum I distinct but short, both free from metathorax; coxosternum without midventral sac. Small spiracles I to VIII located in pleural membrane, with intrinsic occlusor and extrinsic (coxosternal) dilator muscle. Male: Coxosternum IX (Fig. 2A) not subdivided, forming subgenital lobe with median spatulate process; styli absent. Phallic region (Fig. 2, B and C) with membranous lobes around gonopore and transverse, medially asymmetrically produced sclerite articulating with anterolateral corners of tergum X. Cerci one-segmented, prominent, clasping, not forming differentiated articulation with tergum X. Female: Ovipositor projecting markedly beyond short subgenital lobe formed by coxosternum VIII (Fig. 2D). Gonoplacs short, strongly sclerotized. Gonapophyses VIII markedly blunt distally. Gonapophyses IX largely fused with gonoplacs; composite formation with ventral keel interlocking with dorsal groove on gonapophyses VIII. Gonangulum with usual three articulations. One-segmented cerci much shorter than in male.

Foregut with large proventricle (Fig. 2, E and F) armed with weak, papillose (Fig. 2, G and H) sclerites that terminate in three successive whorls of weakly sclerotized lobes. Midgut caeca a pair of short and wide lateral pouches; no pyriform appendices detected. Heart lacking lateral arteries in mid-abdomen. Abdomen with ventral diaphragm. Abdominal ganglion VII free from terminal ganglion (VIIIff). Egg lacking micropylar plate and (defined) operculum, but with circular ridge (Fig. 2, I and J); chorion with hexagonal pattern of grooves traversed by delicate bars (Fig. 2, K and L).

Genus and species descriptions: *Mantophasma* Zompro, Klass, Kristensen et Adis, gen. nov. Eyes moderately sized, less high than gena. Fore-femora distinctly thickened; mid- and (particularly) hind-femora more slender. Fore- and mid-femora with ventral rows of short spines. Type species by present designation: *Mantophasma zephyra* Zompro, Klass, Kristensen et Adis, sp. nov. Etymology: Latin for west wind. Holotype: Female (Fig. 1, B and C). Label data: D. S. W. Afr. [Namibia] S. G. Seewald "JR. No. 827/09" [possibly meaning specimen 827 from 1909]. Museum für Naturkunde, Humboldt University, Berlin, Germany. Length (frons to epiproct apex), 22.5 mm. Color (as preserved), uniformly light brown. Head (Fig. 1E) rounded, three low tubercles between antennae, no tubercle behind antennae, eyes weakly convex. Other included species: *Mantophasma subsolana* Zompro, Klass, Kristensen et Adis, sp. nov. Etymology: Latin for east wind. Holotype: Male (Fig. 1A). Label data: Tanganyika [Tanzania] Ufipa Dish, L. Kwela, 22.iii.1950, H. O. Backlund. Zoological Museum, University of Lund, Sweden. Length (frons to epiproct apex, specimen shriveled), 17.5 mm. Color, light reddish brown with red spots. Head (Fig. 1D) distinctly angular in anterior view, three

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prominent tubercles between antennae, a small tubercle behind each antenna, eyes strongly convex. It cannot at present be categorically excluded that the *M. zephyra* and *M. subsolana* specimens are conspecific, because the small differences noted could reflect sexual dimorphism. But the wide geographical separation of the findings leads us to consider an assumption of conspecificity to be less cautious than one of specific distinctness.

Raptophasma Zompro, 2001, from Baltic Eocene amber (*7, 8*) bears considerable phenetic similarity to *Mantophasma* and shares with it the apomorphic median projection on the male subgenital plate (Fig. 2A) and the small dorsal process beyond the third tarsomere (Fig. 1, G to I); the arolium is also very similar in the two. *Raptophasma* differs from *Mantophasma* by its larger eyes (higher than gena) and stouter, spineless legs. While these Tertiary fossils can have no bearing on dating the origin of the mantophasmatodean stem lineage (which surely must be much older, because there are records of several lower neopteran orders from at least the Permian), they provide zoogeographic proof that the clade once also occurred in northern Europe.

Mantophasmatodeans are phenetically "orthopteroid" insects, but autapomorphies characterizing known orders in this assemblage ["Polyneoptera" or "lower Neoptera" (*2, 9, 10*)] are lacking. They differ from Phasmatodea (stick insects) by having a hypognathous head; a lack of prothoracic repellent glands (*11*); a different composition of female subgenital lobe (supporting online text), which forms no large operculum (in Phasmatodea covering much of the ovipositor from below); a separation between sterna thorax 3 and abdomen I; and a lack of micropylar plate and operculum in eggs (*12*). They differ from Dictyoptera (the mantis-cockroach-termite clade) by having an unperforated tentorium, a lack of female subgenital lobe from coxosternum VII, coxosternal [rather than tergal (*13, 14*)] origins of abdominal spiracle dilator muscles, and a discrete abdominal ganglion VII (*14*). They differ from Grylloblattodea in their lack of metaspina (plesiomorphy or apomorphy?), lack of membranous sac on abdominal venter I, and presence of musculated closing devices of abdominal spiracles (*15*); they differ from Dermaptera (earwigs) in their elongate coxae and lack of female subgenital lobe from coxosternum VII; and they differ from both Grylloblattodea and Dermaptera by having a hypognathous head. Although wingless and nonjumping Orthoptera (grasshoppers and their relatives) can be modified almost beyond recognition, the large prothoracic pleuron unconcealed by pronotal lobes and the lack of an anterior intervalvula in the ovipositor exclude *Mantophasma* from this clade. The lack of longitudinal series of denticles in the proventricle and the full number of tarsomeres (five) are incompatible with *Mantophasma* being subordinate in crown group Ensifera (long-horned grasshoppers) or Caelifera (short-horned grasshoppers). The well-developed ovipositor and the discreteness of basal tarsomeres exclude *Mantophasma* from Plecoptera (stoneflies).

The small process beyond the third tarsomere (Fig. 1, G to I) and the distinctive course of the subgenal suture (Fig. 1E; not known in *Raptophasma*) are putative autapomorphies of the Mantophasmatodea.

Pterygota (winged insects) share the apomorphic absence of a noncuticular trunk endoskeleton and of the lateral parts of abdominal tergum XI [epiproct remaining (*16*)]. Mantophasmatodea exhibit the same features, and a position in the Pterygota-Neoptera is suggested by the valve-like shape of the gonoplacs, their lack of styli, and the apodemes of the abdominal spiracles (*16*). We have found no evidence for a close relationship of Mantophasmatodea to the Acercaria or Endopterygota. Some apomorphies of Mantophasmatodea are recurrent in various Neoptera but are strongly homoplastic. These include a lack of epistomal sulcus, ocelli, and male styli; cutting mandibles; basal tarsomere immobilization; and one-segmented cerci. Evidence that Mantophasmatodea are the closest relatives of Phasmatodea and/or Grylloblattodea appears to deserve particular attention, but it too is ambiguous.

The very large arolium of Mantophasmatodea (Fig. 1J) strikingly resembles that in the basal phasmatodean *Timema* (*11*). However, arolia are smaller in other stick insects, hence the enlargement may not be the ordinal groundplan state. *Mantophasma* (Fig. 2E) and Grylloblattodea (*17*) display a similar configuration of sclerites and lobes in the proventricle, but any conclusions are ham-

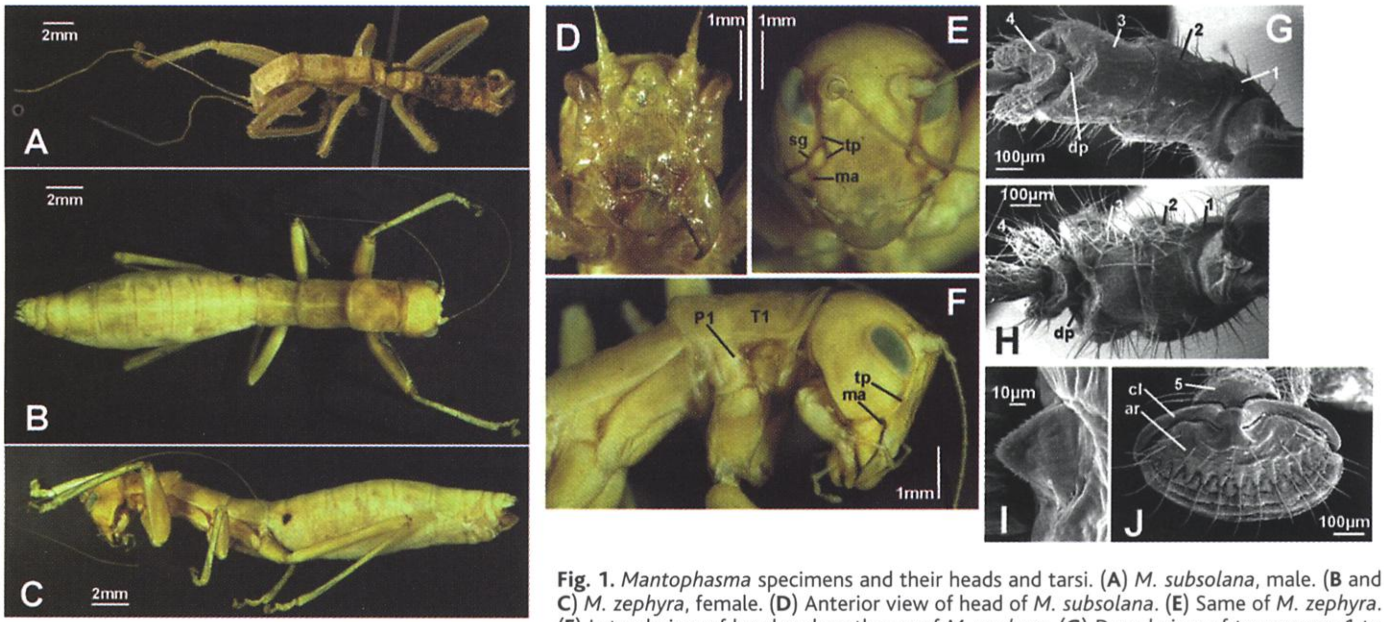

Fig. 1. *Mantophasma* specimens and their heads and tarsi. (**A**) *M. subsolana*, male. (**B** and **C**) *M. zephyra*, female. (**D**) Anterior view of head of *M. subsolana*. (**E**) Same of *M. zephyra*. (**F**) Lateral view of head and prothorax of *M. zephyra*. (**G**) Dorsal view of tarsomeres 1 to 4 of *M. zephyra*. (**H**) Same of *M. subsolana*. (**I**) Dorsal view of process beyond third tarsomere of *M. subsolana*. (**J**) Arolium of *M. zephyra*. Abbreviations: 1 to 5, tarsomeres 1 to 5; ar, arolium; cl, claw; dp, process beyond third tarsomere; ma, anterior articulation of mandible; P1, pleuron of prothorax; sg, subgenal sulcus; T1, tergum of prothorax; tp, anterior tentorial pit.

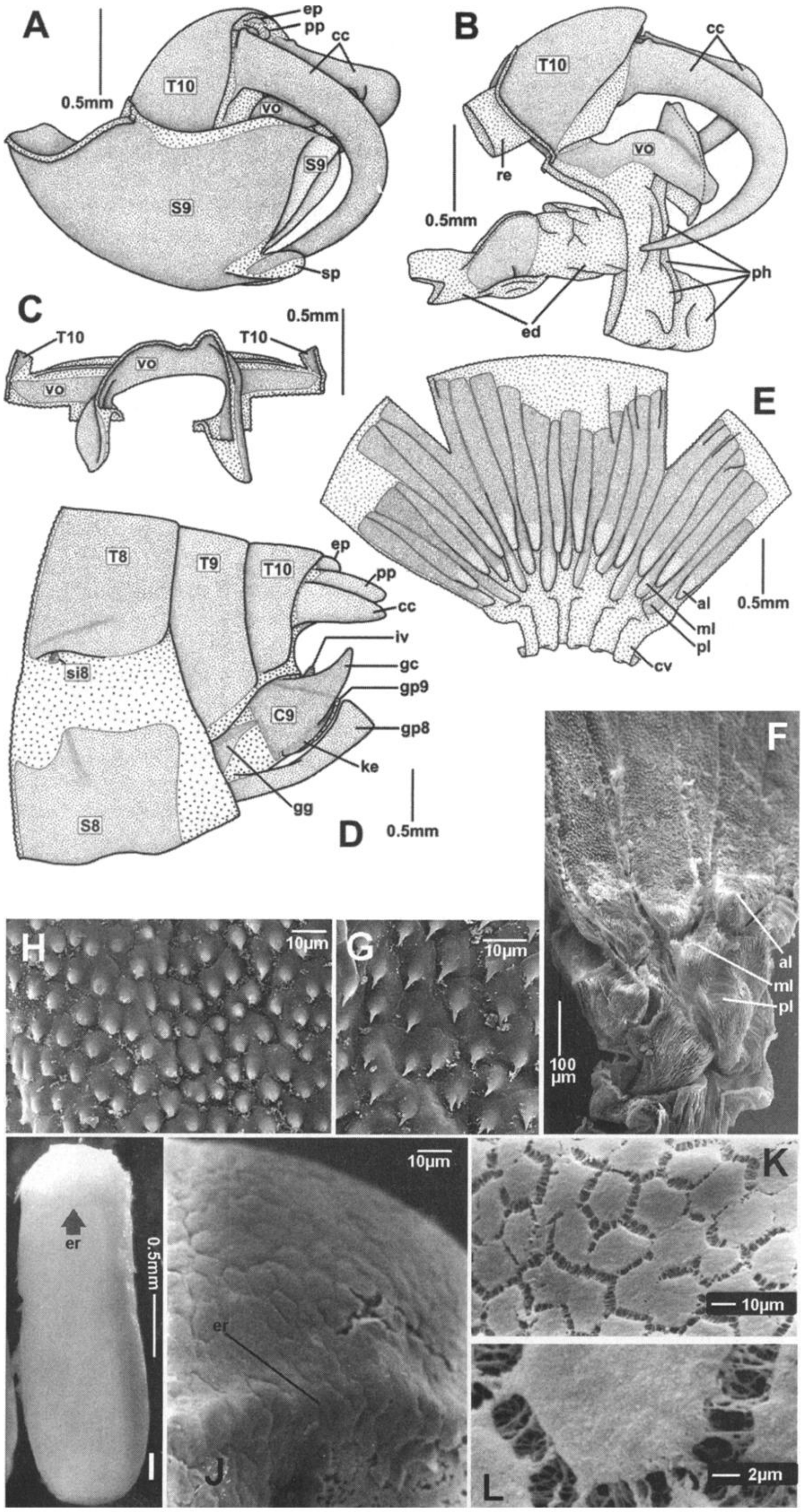

Fig. 2. *Mantophasma* postabdomen, proventricle, and eggs. **(A)** Lateral view of male postabdomen of *M. subsolana*, with subgenital plate (coxosternum IX). **(B)** Same without subgenital plate and with genitalia expanded. Ejaculatory duct opening is between phallic lobes. **(C)** Posterodorsal view of vomer-like element: sclerite, asymmetrical median projection, and lateral articulations with tergum X. **(D)** Lateral view of female postabdomen of *M. zephyra*. **(E)** Exoskeleton of proventricle of *M. zephyra*, viewed from gut cavity. Proventricle is cut longitudinally and spread in a plane, posterior end down. **(F)** Part of proventricle, as seen by scanning electron microscopy. **(G** and **H)** Details of proventricle wall of *M. zephyra*, showing papillose sculpture of proventricle sclerites; papillae in (H) were probably abrased by use. **(I)** Egg of *M. zephyra*, upper end located distally in ovary. **(J)** Chorion around circular ridge. **(K** and **L)** Details of chorion, showing hexagonal grooves with traversing bars. Abbreviations: al, ml, and pl, anterior, middle, and posterior lobes of proventricle; C9, coxa IX; cc, cerci; cv, cardiac valve (entrance into midgut); ed, ejaculatory duct; ep, epiproct; er, circular ridge of egg; gc, gonoplac; gg, gonangulum; gp8 and gp9, gonapophyses VIII and IX; iv, posterior intervalvula; ke, keel inserted into dorsal groove of gonapophysis VIII; ph, phallic lobes (male genitalia s.s.); pp, paraproct; re, rectum; S8 and S9, abdominal coxosterna VIII and IX (subgenital plates); si8, spiracle VIII; T8, T9, and T10, abdominal terga VIII, IX, and X; vo, vomer-like element.

pered by the ambiguous identification of the lobes as teeth or pulvilli (*18*) and homoplasies in the insect proventricle armature. If the lobes are pulvilli, as indicated by their far posterior position, the complete lack of teeth in front of them is apomorphic. If the lobes are the "teeth," as suggested by their shape, their far posterior position is apomorphic. In both cases, the occurrence of sizable lobes upon plicae of three different ranks is apomorphic within insects. However, all these potentially apomorphic conditions are paralleled in other taxa. The condition of the midgut caeca is another possible synapomorphy of Mantophasmatodea and Grylloblattodea (*17*) but is paralleled in many Ensifera, among other taxa. The produced transverse sclerite on segment X in male *Mantophasma* (Fig. 2, B and C) resembles the "vomer" of Phasmatodea (*11, 19*). However, in *Mantophasma* the morphologically posterior rim of the sclerite articulates with tergum X, whereas in phasmids this is true for the anterior rim of the vomer sclerite. Also, the Grylloblattodea have a process-bearing sclerite on venter X; it is, however, restricted to the right side (*15*).

The phylogenetic position of Mantophasmatodea thus remains unclear. A stronger hypothesis will perhaps emerge from anatomical and molecular study of the new material (*20*).

The gut contents of the first discovered museum specimens show the Mantophasmatodea to be carnivores: The midgut and proventricle of both specimens were filled with pieces of arthropod cuticle. As indicated by the rows of short spines along the foreleg and midleg femora and tibiae, prey is captured by means of these legs, as is also done by some Orthoptera (*21*). The chorion structure suggests that the egg is capable of plastron respiration (*22*), as in many insects.

References and Notes

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4. In June 2001, J. Marshall (Natural History Museum, London) showed O.Z. a male insect from Tanzania, which had been submitted for an opinion 16 years ago. Shortly afterward O.Z. received from F. Kernegger a male Baltic-amber insect (subsequently described as *Raptophasma kerneggeri*), whose close similarity to the Tanzania specimen was immediately obvious. In July 2001, O.Z. discovered in the unsorted alcohol collection of Phasmatodea in the Museum für Naturkunde (Berlin) an adult female of a similar insect from Namibia.
5. According to one school of thought among contemporary systematists, the naming of higher taxa that only contain a single genus is "empty formalism"; we accept the logical merits of this stand. Pragmatically we believe, however, that any recognized genus should be assigned to a "family" and an "order," because these categories play an important role in how biologists communicate and how biological knowledge is systematized.
6. The description includes many characters currently known only from a single specimen, because genitalic characters can only be observed in a specimen of the respective sex, muscle characters can only be observed in the ethanol-preserved female, and only a few characters can be judged in the *Raptophasma* fossils.
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20. Since the completion of the work described here, additional (including live) material of further mantophasmatodean taxa has been observed and collected in Namibia by O.Z. and colleagues during an international expedition supported by the Max-Planck-Institut für Limnologie (Plön, Germany), the National Museum of Namibia (Windhoek, Namibia), and Conservation International (Washington, D.C.).
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23. We thank the Museum für Naturkunde, Humboldt-University, Berlin (M. Ohl, I. Dorandt), the Natural History Museum, London (J. Marshall), and the Zoological Museum, University of Lund (R. Danielsson) for assistance and permission to study the *Mantophasma* specimens; G. Brovad and R. Meier (Zoological Museum, Copenhagen) for help in photographic documentation; and O. Kraus (Zoologisches Institut und Zoologisches Museum, Universität Hamburg), W. Dohle (Institut für Zoologie, Freie Universität Berlin), S. Golovatch (Russian Academy of Sciences, Moscow), H. Enghoff (Zoological Museum, Copenhagen), and T. L. Erwin (Smithsonian Institution, Washington, D.C.) for comments on the manuscript. This publication is dedicated to Professor W. J. Junk (Max-Planck-Institut für Limnologie, Plön).

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Pyrrolysine Encoded by UAG in Archaea: Charging of a UAG-Decoding Specialized tRNA

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Pyrrolysine is a lysine derivative encoded by the UAG codon in methylamine methyltransferase genes of *Methanosarcina barkeri*. Near a methyltransferase gene cluster is the *pylT* gene, which encodes an unusual transfer RNA (tRNA) with a CUA anticodon. The adjacent *pylS* gene encodes a class II aminoacyl-tRNA synthetase that charges the *pylT*-derived tRNA with lysine but is not closely related to known lysyl-tRNA synthetases. Homologs of *pylS* and *pylT* are found in a Gram-positive bacterium. Charging a tRNA_{CUA} with lysine is a likely first step in translating UAG amber codons as pyrrolysine in certain methanogens. Our results indicate that pyrrolysine is the 22nd genetically encoded natural amino acid.

In *Methanosarcina* species, specific methyltransferases initiate methanogenesis and carbon assimilation from substrates such as trimethylamine (TMA), dimethylamine (DMA), or monomethylamine (MMA). The highly expressed, nonhomologous genes encoding these methyltransferases have in-frame UAG (amber) codons that do not stop translation during synthesis of the full-length proteins (*1–3*). Nearly identical copies of the DMA and MMA methyltransferase genes with conserved single in-frame amber codons occur in the same genome (*2*, *4*). There is no evidence for transcript editing (*2*, *4*), and, unlike many other stop codon readthrough events (*5*), readthrough of the amber codons is highly efficient (*3*). Amber serves as a sense codon within the methylamine methyltransferase genes (*3*), previously unknown in any other group of archaeal genes. In the accompanying manuscript, Hao *et al.* have shown that in intact MtmB the amber-encoded residue is pyrrolysine, whose structure is proposed as lysine with its epsilon nitrogen in amide linkage with (4*R*,5*R*)-4-substituted-pyrroline-5-carboxylate. Here, we describe a specialized tRNA_{CUA} and lysyl-tRNA synthetase (LysRS) that underlie amber codon translation as pyrrolysine in certain methane-producing Archaea.

An unannotated gene, *pylT* (Fig. 1), whose predicted tRNA product has a CUA anticodon, was identified in the *Methanosarcina barkeri* Fusaro genomic database (GenBank accession number NC_002724) using tRNAScanSE (*6*, *7*). Sequencing of *M. barkeri* MS DNA (*7*) also revealed *pylT*, as well as the three following open reading frames, *pylS*, *pylB*, and *pylC* (GenBank AY064401). Northern blots of the RNA pool from MMA-grown *M. barkeri* MS revealed an RNA of the size expected for the tRNA_{CUA} product of *pylT* (Fig. 1C). The predicted secondary structure of tRNA_{CUA} has unusual properties compared with typical tRNAs (*8*). Even though the structure has the expected sizes for the acceptor, D, and T stems and D, T, and anticodon loops, the anticodon stem could form with six, rather than five, base pairs. This would constrain the variable loop to only three, rather than four, bases. However, if the anticodon stem has five base pairs, two bases are found between the D and anticodon stems, also atypical of most tRNA structures. The predicted secondary structure of tRNA_{CUA} has only one base, rather than the typical two bases, between the acceptor and D stems. Many of the conserved bases in tRNAs are found, but not the almost universally conserved GG sequence in the D loop or the TψC sequence in the T loop.

A 4.2-kilobase transcript was detectable with probes for *pylT*, *pylS*, *pylB*, and *pylC* (Fig. 1D), indicating possible cotranscription of these genes. The *pylS* gene has a predicted product similar to the core catalytic domains of class II aminoacyl-tRNA synthetase (AARS) enzymes containing motifs 1 through 3 (*9*, *10*). An RPS-BLAST (*11*) search for conserved domains found in the Pfam database (*12*) maintained at the National Center for Biotechnology Information produced alignments of different portions of the predicted PylS sequence with class II AARS from three different subclasses (*13*, *14*). To test the activity of PylS as an AARS, the *M. barkeri pylS* gene was expressed in *Escherichia coli* (*7*) as a 49-kD protein with an NH_2-terminal hexahistidine tag, which was then isolated by nickel-affinity chromatography (Fig. 2A). The recombinant PylS was tested for LysRS activity, as pyrrolysine is a lysine derivative. PylS ligated [^{14}C]lysine,

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