Four New Species of Oswaldozruzia (Nematoda: Trichostrongylina, Molineoidea) Parasitizing Amphibians and Lizards from Ecuador

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Description of four new species of Oswaldozruzia parasitizing Iguanidae and Leptodactylidae from Ecuador, demonstrate that they are morphologically close to each other. Like most of the other neotropical and holarctic Oswaldozruzia, they are characterized by spicules with three main branches: blade, shoe and fork; the division of the fork within the distal third of the spicule length appears to be characteristic of the neotropical species.

- Oswaldozruzia bainae n. sp. parasitizing Anolis chrysolepis and Anolis fuscoauratus possesses a synlophe visible only on transversal sections of the body. It is composed of rounded and not pointed ridges.

- Oswaldozruzia tcheprakovae n. sp. parasitizing Eleutherodactylus altamazonicus is closely related to O. bainae, but the synlophe is present only in the anterior and posterior extremities of the body.

- Oswaldozruzia cassonei n. sp. parasitizing Eleutherodactylus lanthanites is closely related to O. taranchoni, Ben Slimane and Durette-Desset, 1995, a parasite of Bufo marinus from Brazil. It is differentiated by the synlophe and the measurements.

- Oswaldozruzia petterae n. sp. parasitizing Leptodactylus pentadactylus is closely related to O. chambrieri, Ben Slimane and Durette-Desset, 1993, parasitizing Bufo and Eleutherodactylus in the same region. It is differentiated since, for an equivalent length of the body, the ridges are almost two times fewer and the spicules smaller.

Key words: Oswaldozruzia n. spp.- Nematoda - Trichostrongylina - Iguanidae - Leptodactylidae - Ecuador

In this study, we continue the review of the genus Oswaldozruzia Travassos, 1917, a cosmopolitan parasite of Amphibians and Reptiles. The diagnosis of the species relies on new morphological criteria, particularly the synlophe characteristics in oesophageal region, the relative arrangement of rays 6 and 8 of the caudal bursa and the acute spicular morphology.

Six species of Oswaldozruzia were described in Ecuador (cf Ben Slimane & Durette-Desset, 1993, 1995), but only from Amphibians. We describe below three new species in Amphibians and one in Reptiles.

MATERIALS AND METHODS

The Nematodes were collected in the small intestine of Ecuadorian Leptodactylidae and Iguanidae (neotropical fauna, guyano-brazilian sub-zona).

The study of the synlophe is based on the Durette-Desset (1985) method; the nomenclature of the synlophe in oesophageal region follows Ben Slimane et al. (1993). More particularly, the cervical alae are defined as one or more latero-ventral ridges, more developed than the other ridges. The nomenclature of the caudal bursa follows Durette-Desset and Chabaud (1981), concerning the relative arrangement of rays 6 and 8 follows that of Durette-Desset et al. (1992). The spicules were studied after dissection and the nomenclature is that of Ben Slimane et al. (1993).

The material was stored in 70° ethanol and deposited in the Helminthological Collections of the Muséum National d’Histoire Naturelle of Paris (MNHN) and in those of the Muséum d’Histoire Naturelle de Genève (MHNG).

DESCRIPTION

The species are closely related to each other and to the other species previously studied in the same region. Some characters do not provide specific differences and can be defined similarly for all the species.
Head: cephalic vesicle present without anterior swelling. *En face* view: buccal aperture triangular, with 6 externo-labial papillae, 4 cephalic papillae and 2 amphids. Small dorsal oesophageal tooth present.

Anterior extremity: excretory pore situated within distal third of oesophagus. Triangular-shaped deirids, posterior to excretory pore. Well-developed excretory glands. Musculo-glandular separation of oesophagus acutely visible at nerve ring level.

Caudal bursa: with 2-3 pattern which tends towards 2-1-2 i.e. extremities of rays 4 directed towards anterior of body, nearer those of rays 3 than rays 5. Rays 2 and 3 joined along, rays 5 and 6 joined along. Rays 8 arising on root of dorsal ray and overlapped by rays 6 except in their distal extremity (type III). Rays 9 arising on dorsal ray before division of the latter into two branches of which internal ones are longest. Thick dorsal ray. Gubernaculum absent.

Genital cone: 15 µm high by 15 µm wide at its proximal part, bearing on anterior lip a large papilla “zero” and 2 min papillae 7 on posterior lip. Spicules: divided proximally into three main branches: externo-lateral branch or blade, interno-dorsal branch or shoe, interno-ventral branch or fork. Fork divided within distal third of spicule. Female: didelphic with very short infundibula.

**Oswaldocruzia bainae** n. sp.

Type-material: holotype male, allotype female MNHN-INVE 19484; 1 male, 3 females paratypes MNHN 212 MD

Host: *Anolis chrysolepis* (Iguanidae)

Site: small intestine

Locality: San Pablo, Ecuador

Voucher specimens: from the same site and the same locality as the types

In 9 *Anolis chrysolepis*: 11 males, 5 L4 males, 16 females, 10 L4 females, MHNG-INVE 19485 to 19491. 7 male, 1 L4 male, 9 females, 1 entsheathed LA female, 2 L4 females MNHN 206 MD-207 MD. In 6 *Anolis fuscoauratus*: 12 males, 3 females: MHNG-INVE 19492-19497; 2 males, 4 females: MNHN 219 MD.

**ADULTS**

Small nematodes with anterior part of body coiled or not. Cervical alae absent. Synlophes: (studied in 1 male and 1 female paratypes, 1 male and 1 female from *A. chrysolepis* 2 males from *A. fuscoauratus*. Numbers in brackets correspond to voucher specimens).

In both sexes, cuticle bears longitudinal “pseudoridges” or undulations, uninterrupted whole length of body but obvious only in transversal sections (Fig. 1C). In male 65% (74 to 77%) of ridges appear in oesophageal region, within 67% (74 to 78%) of dorsal ridges and 63% (71 to 81%) of ventral ridges. In female 81% (71%) of ridges appear in oesophageal region within same dorsal and ventral ratio. Ridges disappear just anterior to caudal bursa in male and at phasmids level in female.

In male 27 (30, 34, 26) ridges at oesophago-intestinal junction (Fig. 1C) and 40 (40, 44, 35) at mid-body (Fig. 1D). In female, 44 (34) ridges at oesophago-intestinal junction and 54 (48) at mid-body (Fig. 1E).

In transversal sections, rounded ridges are more or less regularly spaced.

Holotype-male: 7100 µm long and 130 µm wide at mid-body. Cephalic vesicle 75 µm long and 40 µm wide. Nerve ring, excretory pore and deirids 190 µm, 380 µm and 400 µm from apex, respectively. Oesophagus 530 µm long.

Caudal bursa illustrated in Fig. 1K. Spicules 190 µm long, blade divided at its distal part into two small branches which are subdivided into numerous processes; fork distally divided at 17% of whole length of spicule (Fig. 1H-J).

Allotype-female: 12350 µm long and 190 µm wide at mid-body. Cephalic vesicle 90 µm long and 40 µm wide. Nerve ring, excretory pore and deirids 190 µm, 370 µm and 390 µm from apex, respectively. Oesophagus 540 µm long (Fig. 1A).

Vulva 4150 µm from caudal extremity, vagina vera 50 µm dividing vestibule 380 µm long into two equivalent parts. Sphincters both 40 µm long and infundibula both 30 µm long (Fig. 1F). Uterine branches both 2450 µm long with 70 eggs. All eggs at morula stage, 70 µm long and 50 µm wide. Tail 160 µm long and 70 µm wide at level of anus, with caudal spine 16 µm long (Fig. 1G).

**ENTSHEATHED IMMATURE STAGES**: (studied in 1 male and 1 female from *A. chrysolepis*).

Male: 3200 µm long and 90 µm wide at mid-body. Nerve ring, excretory pore and deirids 170 µm, 310 µm and 330 µm from apex, respectively. Oesophagus 390 µm long; beginning of formation of bursate rays (Fig. 2E).

Female: 4100 µm long and 90 µm wide at mid-body. Nerve ring, excretory pore and deirids 150 µm, 250 µm and 250 µm from apex, respectively. Oesophagus 370 µm long; tail 150 µm long and 40 µm wide at level of anus with caudal spine 18 µm long.

Synlophes: same shape as in adult (Fig. 2D).

**4TH STAGE LARVA**: (studied in 1 male and 1 female from *A. chrysolepis*).

Head: cephalic vesicle absent (Fig. 2A).

Synlophes: in both sexes composed of two lateral
Fig. 1: Oswaldocruzia bainae n. sp. from Anolis chrysolepis: Adults - A: female, anterior extremity, left lateral view. B: male, head, apical view. C to E: synlophe in transversal sections. C: male, at oesophago-intestinal junction. D: male, at mid-body. E: female, id. F: female, ovejector, left lateral view. G: female, tail, left lateral view. H to J: male, dissected left spicule. H: ventral view. I: dorsal view. J: externo-lateral view. K: male, caudal bursa, ventral view. All the sections are orientated as C. A, F, G bar = 60 µm; C, D, E bar = 40 µm; B bar = 20 µm; H, I, J bar = 30 µm; K bar =50 µm. Abbreviations: d. = dorsal; v. = ventral; l. = left; r. = right. de.= deirid; d.o.t = dorsal oesophageal tooth; f = fork; s = shoe; b = blade.
alae held by chitinous skeleton whole along the body (Fig. 2C).

Male: 3400 μm long and 80 μm wide at mid-body.
Nerve ring, excretory pore and deirids 175 μm, 300 μm and 320 μm from apex, respectively. Oesophagus 390 μm long. Genital apparatus 1450 μm long.

Female: 3250 μm long and 70 μm wide at mid-body.
Nerve ring, excretory pore and deirids 150 μm, 240 μm and 240 μm from apex, respectively. Oesophagus 370 μm long (Fig. 2A). Genital apparatus 610 μm long (Fig. 2G). Tail 110 μm long by 30 μm wide at anus level (Fig. 2F).

**DISCUSSION**

The specimens from Ecuadorian *A. chrysolepis* and *A. fuscoauratus* have no specific differentiation each other and belong to the genus *Oswaldocruzia* Travassos, 1917.

Among the numerous known species in the genus, those which present, as Ecuadorian specimens, spicules divided proximally into three main branches with a fork distally divided (within the distal third) seem characteristic of the neotropical zone.

The specimens described above possess: (1) a relative arrangement of the rays 6 and 8 of type III, i.e. the rays 8 arise on root of the dorsal ray and are overlapped by the rays 6 except at their distal extremity; (2) a spiculic blade distally divided into numerous processes; (3) a poor developed synlophe with undulations and not sharp ridges.

Only one other neotropical species has the same synlophe: *O. peruensis* Ben Slimane et al. (1995), a parasite of Peruvian *Stenocercus roseiventris*. It differs from the Ecuadorian specimens by the relative arrangement of the rays 6 and 8 (type II) and by the presence of the cervical alae.

We consider the specimens from *Anolis* spp. as belonging to a new species *Oswaldocruzia bainae* n.sp., named after our colleague, Dr Odile Bain.

*Oswaldocruzia tcheprakovae* n.sp.

Type-material: holotype male, allotype female MHNG-INVE 19506, 1 L4 male, 5 female paratypes MNHN, 178 MD

Host: *Eleutherodactylus altamazonicus* (Leptodactyliidae)

Site: small intestine

Locality: San Pablo, Ecuador

**ADULTS**

Small nematodes, with anterior part of body coiled.

Synlophe: (studied in the holotype and 1 paratype female). In both sexes, absence of synlophe except in anterior and posterior extremities of body where ridges longitudinal. According to sex and level of body, ridges are rounded (undulations) or pointed.

In anterior extremity, ridges appear behind cephalic vesicle. In male, dorsal ridges disappear at about 640 μm from apex and ventral ones at about 700 μm. In female (7000 μm long), dorsal ridges disappear at about 1200 μm from apex (i.e three times length of oesophagus) and ventral ones at about 2400 μm (i.e a third of whole length). In posterior extremity, dorsal ridges of male (3650 μm long) visible at about 1400 μm from caudal bursa in transversal section of body but *in toto*, only at approximately 450 μm; ventral ridges visible at about 250 μm. In female, ventral ridges appear at ovjector level and dorsal ones at anus level.

In male, 16 dorsal ridges at 1400 μm to caudal bursa (Fig. 3I). In female, 26 ridges (13 dorsal, 13 ventral) at oesophago-intestinal junction (Fig. 3B), 13 ventral ridges at ovjector level (Fig. 3G) and 21 ridges at anus level (Fig. 3H).

In transversal section, pointed ridges are orientated perpendicularly to body surface. Undulations and ridges are both with same size and irregularly spaced. Holotype-male: 3650 μm long and 70 μm wide at mid-body. Cephalic vesicle 50 μm long and 30 μm wide. Nerve ring, excretory pore and deirids 140 μm, 230 μm and 250 μm from apex, respectively. Oesophagus 370 μm long.

Caudal bursa illustrated in Fig. 4H. Spicules not dissected, 130 μm long. Blade divided at its distal part into two small branches. Subdivision of small branches not seen. Fork distally divided at 28% of whole length of spicule.

Allotype-female: 7350 μm long and 100 μm wide at mid-body. Cephalic vesicle 50 μm long and 30 μm wide. Nerve ring, excretory pore and deirids 150 μm, 280 μm and 300 μm from apex, respectively. Oesophagus 400 μm long (Fig. 4A).

Vulva 2600 μm from caudal extremity. *Vagina vera* 30 μm long dividing vestibule 180 μm long into two parts, posterior one being slightly shorter. Sphincters both 30 μm long and infundibula both 25 μm long (Fig. 4D). Anterior uterine branch 1800 μm long with 31 eggs, posterior uterine branch 1700 μm long with 28 eggs. All eggs at morula stage 70 μm long and 40 μm wide. Tail 140 μm long and 50 μm wide at level of anus, with caudal spine 15 μm long (Fig. 4C).

**4TH STAGE LARVA MALE:** 2000 μm long and 50 μm wide at mid-body. Head without cephalic vesicle. Nerve ring, excretory pore and deirids 110 μm, 180 μm and 190 μm from apex, respectively. Oesophagus 360 μm long. Synlophe composed of two lateral ridges orientated perpendicularly to body surface and supported by a chitinous skeleton (Fig. 4K).
Fig. 2: *Oswaldocruzia bainae* n. sp from *Anolis chrysolepis*: 4th stage larvae and entsheathed immature stages (Im.) - A: L4 female, anterior extremity, right lateral view. B to D: synlophes in transversal sections. B: (Im.) female, at oesophago-intestinal junction, the immature synlophes is not yet formed. C: L4 male, at mid-body. D: (Im.) female, at mid-body, showing both L4 and (Im.) synlophes. E: (Im.) male, caudal bursa of the adult inside the tail of the L4, left lateral view. F: L4 female, tail, right lateral view. G: L4 female, genital apparat showing the beginning of the differentiation between the ovejector, the uterine branches and the ovaries, left lateral view. All sections are orientated as B. A, F bar = 50 μm; B to G bar = 40 μm. Abbreviations: d. = dorsal; v. = ventral; l. = left; r. = right; (Im.) = entsheathed immature in L4.

**DISCUSSION**

With a synlophes mainly composed of rounded and not pointed ridges, the species can be related to *O. peruensis* Ben Slimane et al. (1995) parasite of *Sternocercus roseiventris* in Peru and *O. bainae* n.sp. parasite of *Anolis* spp. It is closely related to the latter since the specimens described above, have an arrangement of rays 6 and 8 of type III and no cervical alae as *O. bainae*.

But the specimens of *Eleutherodactylus altamazonicus* can be distinguished from all the neotropical species by its very particular synlophes lacking ridges in the median part of the body.

We therefore consider the specimens from *Eleutherodactylus altamazonicus* as belonging to...
Fig. 3: *Oswaldocruzia tcheprakovae* n.sp. from *Eleutherodactylus altamazonicus*. A-I: synlophe in transversal sections. A-H: female (7000 μm long). A: at excretory pore level. B: at oesophago-intestinal junction. C: at 830 μm from apex (twice the length of oesophagus). D: at 1180 μm from apex. E: at 2360 μm from apex, at about level of the first third of body. F: at mid-body. G: at ovejector level. H: at anus level. I: male, at 1400 μm from the caudal bursa. J-L: L4 male. J: head, right lateral view. K: synlophe at mid-body. L: tail, right lateral view. All the sections are orientated as B. Abbreviations: d. = dorsal; v. = ventral; l. = left; r. = right.
Fig. 4: Oswaldocruzia tcheprakovae n.sp. from Eleutherodactylus altamazonicus. A-D: female - A: anterior extremity, left lateral view. B: head, apical view. C: tail, left lateral view. D: ovjector, left lateral view. E-H: male. E: caudal bursa, showing the disappearance of the ventral ridges. F: anterior extremity, showing the appearance and the disappearance of the ridges, right lateral view. G: posterior extremity, showing the appearance and the disappearance of the ridges, right lateral view. H: caudal bursa, ventral view. A, C, D bar = 40 µm; B bar= 20µm; E bar = 50 µm; F, G bar = 60 µm; H bar = 30 µm. Abbreviations: de.= deirid; d.o.t= dorsal oesophageal tooth.
a new species *Oswaldocruzia tcheprakova* n.sp. named after our colleague Roselyne Tcheprakov.

**Oswaldocruzia cassonei** n.sp.

Type-material: holotype male, allotype female MHNG-INVE 19477, 4 males, 1 female MNHN, 169 MD

Host: *Eleutherodactylus lanthanites* (Leptodactylidae)

Site: small intestine

Locality: San Pablo, Ecuador

Voucher specimens: from the same site and the same locality, as the types

In 5 *E. lanthanites*: 3 males, 3 females MHNG-INVE 19478-79, 3 males MNHN 170 MD-172 MD. In 2 *E. conspicillatus*: 4 males MHNG-INVE 19480-81, 3 males MNHN 174 MD. In 1 *E. diadematus*: 1 female MHNG-INVE 19482. In 1 *E. altamazonicus*: 1 male MHNG-INVE 19483.

**ADULTS**

Small nematodes, curved on ventral line. Cervical alae absent.

Synlophe: (studied in 2 male and 1 female paratypes, 2 males, parasites of *E. lanthanites* and 1 male, parasite of *E. conspicillatus*). Numbers in brackets correspond to voucher specimens.

In both sexes, cuticle bears uninterrupted longitudinal ridges. In male, 85-93% of ridges appear in oesophageal region within 79-100% of dorsal ridges and 87-100% of ventral ridges. In female 86% of ridges appear in oesophageal region within same dorsal and ventral ratio. Ridges disappear just anterior to caudal bursa in male and at phamids level in female.

In male, 29, 31 (28, 28, 26) ridges at oesophago-intestinal junction (Fig. 5C) and 32, 35 (30, 33, 34) ridges at mid-body (Fig. 5E). In female, 36 ridges oesophago-intestinal junction (Fig. 5D) and 42 ridges at mid-body (Fig. 5F).

In transversal section, ridges same size, orientated perpendicularly to body surface with regular spacing.

Holotype male: 4200 µm long and 100 µm wide at mid-body. Cephalic vesicle 50 µm long and 30 µm wide. Nerve ring, excretory pore and deirids 130 µm, 210 µm and 230 µm from apex, respectively. Oesophagus 380 µm long (Fig. 5A). Caudal bursa illustrated in Fig. 6M. Spicules 125 µm long. Blade with spatulate extremity, fork distally divided at 23% of whole length of spicule (Fig. 5 H, I, J).

Allotype-female: 9200 µm long and 130 µm wide at mid-body. Cephalic vesicle 65 µm long and 35 µm wide. Nerve ring, excretory pore and deirids 160 µm, 270 µm and 290 µm from apex, respectively. Oesophagus 480 µm long.

Vulva 2850 µm from caudal extremity. *Vagina vera* 30 µm long dividing vestibule 220 µm long into two equivalent parts. Sphincters both 30 µm and infundibula both 20 µm long (Fig. 5G). Anterior uterine branch 1900 µm long with 38 eggs, posterior uterine branch 1800 µm long with 26 eggs. All eggs at morula stage, 70 µm long and 50 µm wide. Tail 120 µm long and 50 µm wide at level of anus with caudal spine 15 µm long (Fig. 5K).

**DISCUSSION**

In the neotropical region, the sole species closely related to the specimens described above is *Oswaldocruzia taranchoni* Ben Slimane and Durette-Desset (1995) a parasite of *Bufo marinus* from Pernambuco, Brazil which has both the caudal bursa of type III and the spicular blade with spatulate extremity. Unlike the specimens described above, in *O. taranchoni*, the sexual dimorphism concerning the size is slighter (male 6.4-5 mm, female 7.35 mm), the ridges are more numerous (53-75 at mid-body in females) and not pronounced, and the division of the spicular fork is deeper. We consider the specimens from *Eleutherodactylus* as belonging to a new species *Oswaldocruzia cassonei* n.sp named after our colleague Jimmy Cassone.

**Oswaldocruzia petterae** n.sp.

Type-material: holotype male, allotype female MHNG-INVE 19500, 1 male, 1 female paratypes MNHN 153 MD

Host: *Leptodactylus pentadactylus* (Leptodactylidae)

Site: small intestine

Locality: San Pablo, Ecuador

Voucher material: from the same site and the same locality as the types

In 9 *L. pentadactylus*: 6 males, 5 females, 1 L4 female, MHNG-INVE 19501-505; 3 males, 3 females, MNHN 148 MD, 149 MD, 154 MD, 155 MD.

**ADULTS**

Small nematodes, with anterior part of body coiled. Cervical alae absent.

Synlophe: (studied in the male and the female paratypes; in 2 males and 2 females from voucher material. Numbers in brackets correspond to voucher specimens).

In both sexes, cuticle bears longitudinal ridges over whole length of body. In male 82-100% of ridges appear in oesophageal region within 87-100% of dorsal ridges and 77-100% of ventral ridges. In female 86% of ridges appear in oesophageal region within same dorsal and ventral ratio. Ridges
disappear just anterior to caudal bursa in male and at phasmids level in female. In male, 23 (26, 27) ridges at oesophago-intestinal junction (Fig. 6C) and 28 (26, 30) at mid-body (Fig. 6E). In female, 34 (33, 31) ridges at oesophago-intestinal junction (Fig. 6D), and 38 (41, 38) at mid-body (Fig. 6F).

In transversal section, ridges are oriented perpendicularly to body surface, same size and regularly spaced except in oesophageal region where the ridges in front of lateral fields are more spaced. Holotype-male: 5000 µm long and 90 µm wide; cephalic vesicle 65 µm long and 40 µm wide. Nerve ring, excretory pore and deirids 190 µm long and 350 µm from apex, respectively. Oesophagus 470 µm long (Fig. 6A).

Caudal bursa illustrated in Fig. 6 L. Spicules 140 µm long; blade distally divided into 6 processes, fork distally divided at 21% of whole length of spicule (Fig. 6 I, J, K).

Allotype-female: 9000 µm long and 120 µm wide at mid-body; cephalic vesicle 80 µm long and 35 µm wide. Nerve ring, excretory pore and deirids 200 µm, 350 µm and 370 µm from apex, respectively. Oesophagus 510 µm long Vulva 3100 µm from caudal extremity. Vagina vera 35 µm long dividing vestibule 240 µm long into two equivalent parts. Sphincters both 25 µm long and infundibula both 25 µm long (Fig. 6G).

Anterior uterine branch 2200 µm long with 55 eggs; posterior uterine branch 2200 µm long with 52 eggs. All eggs at morula stage 70 µm long and 50 µm wide. Tail 180 µm long and 65 µm wide at level of anus, with caudal spine 15 µm long (Fig. 6H).

**DISCUSSION**

The specimens from *Leptodactylus* are mainly characterized by (1) a cephalic vesicle without proximal swelling; (2) a synlophe with numerous ridges regularly spaced; (3) the absence of the cervical alae; (4) a caudal bursa of type III and (5) the spicular blades divided into numerous processes at their distal extremities. The only species sharing the same characters is *Oswaldocruzia chambrieri* Ben Slimane and Durette-Desset (1993), a parasite of *Bufo* and *Eleutherodactylus* from the same region as the specimens studied above.

The extra-branches present on the spicular shoes of *O. chambrieri* are not observed in any of the *Leptodactylus* specimens. Therefore, this character is not constant within the same species and cannot be used as a specific character. Only two differences indicate a speciation: the number of ridges at mid-body level and the length of the spicules both in relation to the size of the specimen. In a female of *O. chambrieri*, 9 mm long, the number of ridges is 38 and the length of the spicules is 140 µm. In a 6.4 mm long female parasite of *Leptodactylus*, the number of ridges is 54 and the length of the spicules is 190 µm.

We therefore consider the specimens from *Leptodactylus*, belonging to a new species *Oswaldocruzia petterae* n. sp. named after our colleague Dr Annie Petter.

**REFERENCES**


