

DIPETALONEMA SEMICLARUM SP. NOV.
FROM THE BLOOD OF MAN IN THE REPUBLIC OF ZAIRE
(NEMATODA : FILARIOIDEA)

by

A. FAIN

Summary — A new microfilaria *Dipetalonema semiclarum* sp. nov. is described from the blood of man in Equateur Province, Republic of Zaïre. It has been found in 52 inhabitants of three villages. In Bokela, the most heavily infected village, it was found in 49 per cent of the adult population. The highest number of microfilariae was 210 per thick drop. This microfilaria is unsheathed and subperiodic with a slight peak during the day. It measures $221 \mu \times 5 \mu$ (average length \times width in dermic films with blood) or $198 \mu \times 5.2 \mu$ (average in thick drops). Its general aspect recalls *D. perstans*, but it is larger and in the posterior half of the body there is a long clear band containing few large and scattered nuclei.

KEYWORDS : Filarioidea; Microfilaria; *Dipetalonema semiclarum* sp. nov.; Zaïre Republic.

During a survey on human filariasis in the Province de l'Equateur (= also called « Cuvette Centrale ») in the Republic of Zaïre, we found in the blood of a number of the inhabitants a microfilaria that does not correspond with the description of any known species

The results of this survey have been published elsewhere (Fain, Wéry and Tilkin, 1969). We deal here only with the description of the new microfilaria found on this occasion.

Material and methods

In the course of this survey we examined 800 adults from 10 villages.

Thick drops of blood about 15-17 mm diameter were made by finger prick between 21.00 and 23.00 h.

Dermic fluid, always mixed with blood, was obtained between 10.00 and 12.00 h, by the method of « dermic scarifications » described by D'Hooghe (1934). We have used a slightly modified and standardized technique which allows comparable results in the counting of the skin microfilariae (Fain, Elsen, Wéry and Maertens, 1973). The « dermic scarifications » technique is, in our experience, more accurate in both the diagnosis and counting of skin microfilariae than the skin snips or biopsies more commonly used. By this technique the microfilariae of *Onchocerca volvulus* and *Dipeta-*

TABLE 1
Parasitism by microfilariae in the three infected villages

Village	No. examined (Males : M Females : F)	<i>Wuchereria bancrofti</i> %	<i>Loa loa</i> %	Percentage of infestations				<i>Dipetalonema streptocercum</i> %	<i>Onchocerca volvulus</i> %
				<i>Dipetalonema perstans</i> %	<i>Dipetalonema semiclarum</i> sp. n. %	<i>Dipetalonema perstans</i> %	<i>Dipetalonema semiclarum</i> sp. n. %		
Bokela	M 50	2	1	94	56	80	98		
	F 50	2	0	96	42	60	74		
Wema	M 43	0	4.5	100	2.3	65	93		
	F 64	0	1.5	89	1.5	37	82		
Befale	M 30	0	6.6	76	0	60	40		
	F 71	0	1.4	91	1.4	78	49		

Ionema streptocercum are easily separable and accurately counted, which is not the case in biopsies where the microfilariae are examined in the living stage.

The « dermic scarifications » always contain a certain amount of blood which may vary slightly in quantity, but is generally a little more abundant than in our thick drops. If the technique is carefully performed the quantity of dermic lymph is constant. The dermic lymph mixed with blood is put on a slide where it occupies an area of about 20 mm × 45 mm. Although much larger than a thick drop, the film being thinner, dries more quickly.

The combination of thick drops taken by night and dermic films by day allowed us to detect microfilariae both in the blood (nocturnal and diurnal) and in the skin.

After drying, the slides were directly stained with Giemsa stain (3 drops for 2 ml distilled water) for 45 minutes, without preliminary dehaemoglobinisation or fixation. Dehaemoglobinisation then occurs during staining.

Our material was examined, and the new microfilaria identified, only several months after our return to Belgium. The presence of this new microfilaria was easily recognized in the Giemsa stained slides, but in order to obtain better staining of the nuclei we destained the slides with 70 per cent ethanol and restained them with haematoxylin. We followed the technique described by Fülleborn (1929) and used recently by Schacher (1969).

Results

Distribution and frequency of the new species

This new microfilaria has been found in the blood of 52 Mongo people, inhabitants of three villages in the Province of Equateur (or « Cuvette Centrale »), Republic of Zaïre. The most infected village is Bokela (1°12'S; 22°E), on the river Lomela. In that village 49 per cent of the adults harboured this microfilaria. Infection was more frequent in males (56 per cent) than in females (42 per cent). In the two other villages, Befale (0°29'N; 21°E) and Wema (0°32'S; 21°55'E), the latter situated along the river Tshuapa, infection was very scarce, represented by one and two cases respectively.

Most of the natives of these three villages were also infected by several other microfilariae, as shown in the table 1.

At Bokela the river Lomela is approximately 200 m wide and it flows through the great forest. This river constitutes the northern limit of the Salonga Forest and Game Reserve.

Dipetalonema semiclarum sp. nov.

(Fig. 1-4, 8-12)

Description

The measurements are based on 50 microfilariae in thick drops taken by night and on 30 microfilariae in dermic films containing blood taken in the morning.

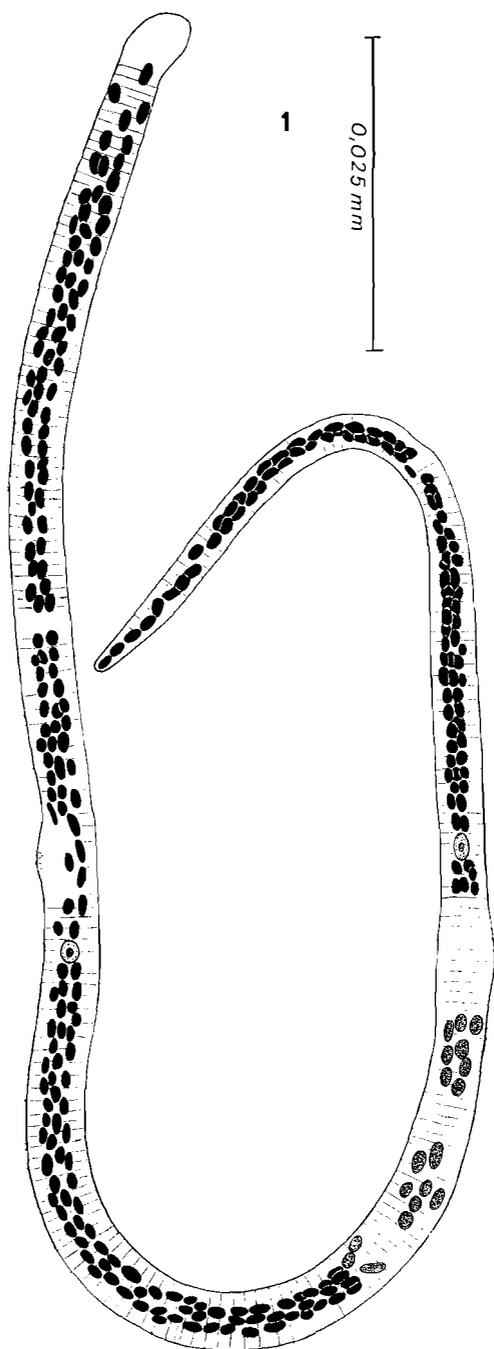
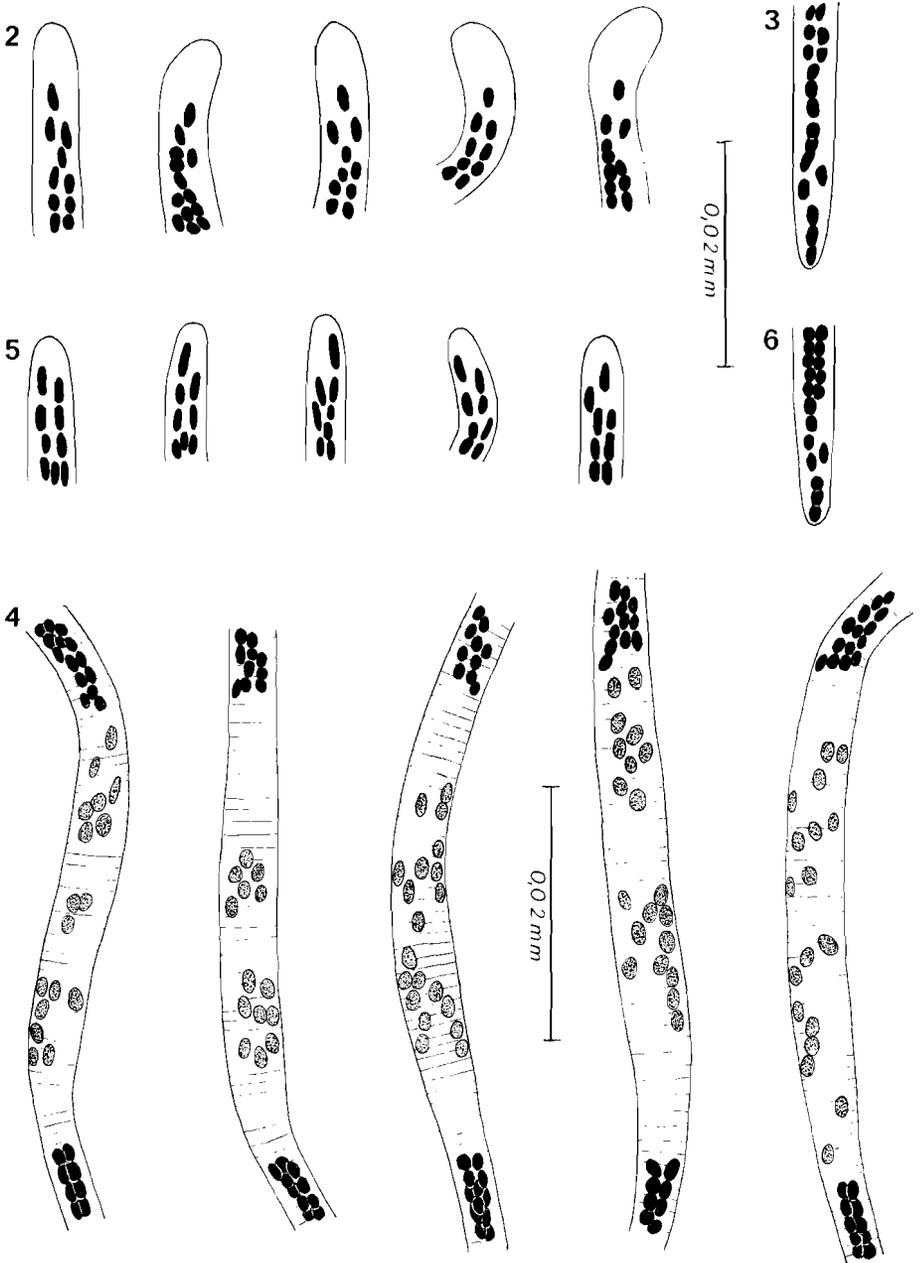


Figure 1

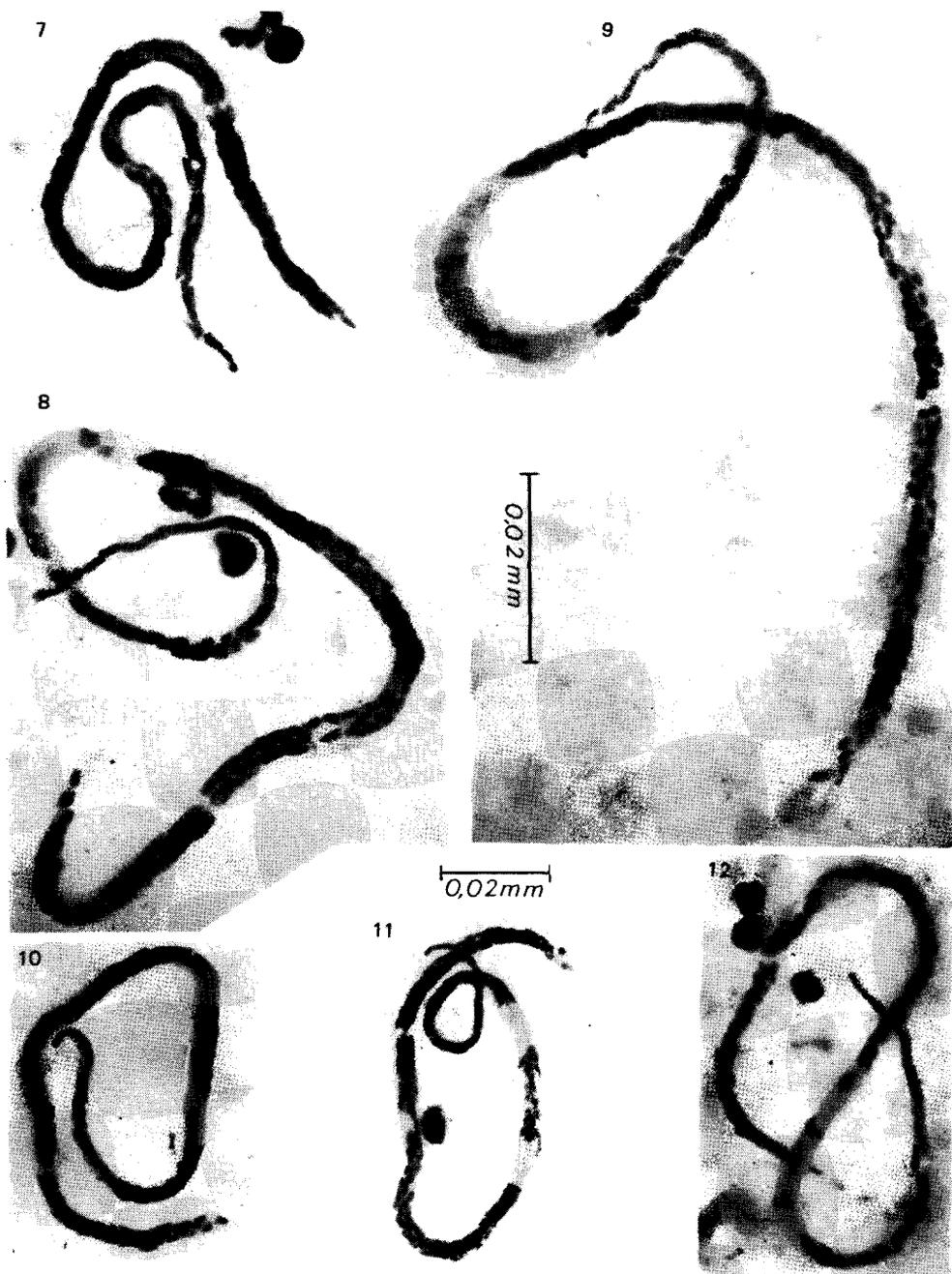
Dipetalonema semiclarum sp. n.



Figures 2-6

Dipetalonema semiclarum sp. n. fig. 2 : various aspects of the head. fig. 3 : tail. fig. 4 : various aspects of the clear region.

Microfilaria of *D. perstans*. fig. 5 : various aspects of the head. fig. 6 : tail (N. B. these microfilariae were present in the same slide as those of *D. semiclarum* sp. n.



Figures 7-12

Microfilariae of *D. perstans* (fig. 7) and of *D. semiclarum* sp. n. (fig. 8) in the same field, showing the differences in size and in morphology between them.

Microfilariae of *D. semiclarum* sp. n. (fig. 9) at the same magnification as n° 7-8
 Microfilariae of *D. semiclarum* sp. n., at a smaller magnification showing « attitudes » and the slight variations in the nuclei of the clear area (fig. 10-12). (N. B. All the microfilariae of these photomicrographs were destained from Giemsa and restained in haematoxylin).

Microfilaria. Unsheathed, subperiodic and a little more numerous in the dermic films taken in the day. Cuticle distinctly striated transversely. Body more loosely coiled than in *D. perstans* and presenting fewer secondary kinks. Head generally slightly swollen and often asymmetrical. The first nucleus is distinctly separated from the other nuclei and it is shorter and broader than in *D. perstans*. Nerve ring and excretory spot always very distinct and relatively long. The main characteristic feature of this species is the presence a little behind the middle of the body of a clear band, measuring 25 to 40 μ (exceptionally more) in length. In this area the nuclei are much less numerous (from 12 to 20) a little larger, less stained and more diffusely distributed than in the rest of the body, they do not form a median column but are scattered and touching the periphery of the body. These nuclei are often grouped in two separated bunches in the median part of the clear area. Beyond this clear area the nuclear column is distinctly smaller than in front of it. Anal spot small. The last part of the nuclear column, about 15 to 20 μ long, is formed by a single often sinuous row of 6 to 10 nuclei, generally longer than wide. The last nucleus is terminal as in *D. perstans*. Somatic nuclei longer than wide, generally overlapping and not countable except in the posterior extremity and for a few specimens in the anterior part of the body. In three specimens, the numbers of the nuclei between the head and the nervous ring were 67, 64 and 62 respectively. Posterior extremity straight, ending in a blunt tip.

Body measurements :

1. *Length*. It varies depending of the mode of sampling. In all the thick drops taken by night, the length is approximately one tenth smaller (mean 197.6 μ , range 184-220 μ) than in the dermic films taken by day (mean 221.1 μ , range 203-242 μ). The shortening and the more contracted aspect of the microfilariae in the thick drops taken by night, in comparison with those of the dermic film taken by day, may be explained by the fact that these samples dried more slowly owing to the greater thickness of the thick drop and the lower temperature prevailing at night. Fulleborn (1929) has already drawn attention to this phenomenon.

In the slides containing *D. semiclarum* we found numerous microfilariae of *D. perstans* showing the same variations in length related to the method used. The mean length of these microfilariae is 152 μ (range 140-161 μ) in thick drops taken at night and 169 μ (range 155-180 μ) in dermic films taken in the day (measurements based on 30 microfilariae for both series).

2. *Width*. The mean width is 5.2 μ in the thick drops (range 4.7-5.6 μ) and 5 μ (range 4.6-5.4 μ) in the dermic films. The cephalic space has a width of 3.5 μ (range 2.7-5 μ).

3. *Width of the nuclear column*. The part between the head and the clear band is at its maximum 3.5 μ wide (range 2.9-4 μ) in thick drops and 3.2 μ (range 2.7-3.5 μ) in dermic films. The part between the clear band and the anal spot is at its maximum 2.5 μ wide (mean) in thick drops and 2.2 μ in dermic films. The last part measured a little behind the anus is 1.7 to 1.81 μ wide (mean). The first nucleus is generally shorter (mean 2.2 μ) and wider than in *D. perstans*. The last nucleus is, in some microfilariae, a little thickened as in *D. perstans*, but we believe that it is an artefact related

to a defect of staining, produced by an accumulation of the dye in the tip of the tail.

4. *Excretory cell.* Is situated at 7.4 μ (mean) behind the middle of the excretory spot. We have not clearly seen the genital cells.

5. *Distances from the head of the main organs.* These distances expressed both in microns and as a percentage of the total body length are mentioned in table 3.

Periodicity and intensity of the parasitism

In the slides containing the microfilariae of both *D. semiclarum* and *D. perstans*, the second species was always present in much larger number. *D. semiclarum* was found in relatively greater numbers in the day than in the night samples, which suggests a subperiodicity with a slight diurnal peak. This tendency to daily periodicity is confirmed by the fact that the ratio *D. perstans* : *D. semiclarum* was distinctly greater by night than by day, as shown in table 2.

TABLE 2

Number of microfilariae of *D. perstans* and *D. semiclarum* in nine subjects, in thick drops (TD, by night) and dermic films (DF, by day)

Patients	<i>D. perstans</i>	<i>D. semiclarum</i> sp. n.	Ratio of numbers <i>D. perstans</i> : <i>D. semiclarum</i>
Males :			
N° 2 (TD)	102	28	3.64
(DF)	225	68	3.30
N° 14 (TD)	341	4	85.2
(DF)	412	6	68.6
N° 19 (TD)	182	3	60.6
(DF)	326	12	27.1
N° 20 (TD)	329	21	15.6
(DF)	503	47	10.7
N° 39 (DF)	1,200	210	—
N° 41 (DF)	754	63	—
N° 38 (DF)	437	81	—
Females :			
N° 57 (TD)	135	3	45
(DF)	191	6	31.8
N° 72 (TD)	140	6	23.3
(DF)	70	5	14

Systematic situation of D. semiclarum sp. nov.

D. semiclarum is intermediate in size between *D. perstans* and the larger sheathed species such as *L. loa* and *W. bancrofti*.

It most closely resembles *D. perstans*, but is clearly distinguished from it by the following characters : body length and width significantly greater, shape of the head wider and longer, the first nucleus is shorter, presence in the posterior half of the body of a clear band 25-40 μ long and containing a few nuclei.

D. semiclarum is to be distinguished from two species described in man in Africa during these last 25 years. The first is *Tetrapetalonema berghei* Chardome and Peel, 1951 described from the Republic of Zaïre. The adults of that species have been synonymized with *D. perstans* by Chabaud (1952) and the microfilaria is a little shorter than that of *D. perstans* but by all the other characters is not clearly distinguished from it. The second species is *Microfilaria soudanica* Deschiens and Pfister, 1954, described from Côte d'Ivoire. It is a sheathed microfilaria well characterized by the great thickness (20-21 μ) of the body.

There is a possibility that *D. semiclarum* is a parasite of a wild animal which has become adapted to man. The normal host could be a monkey or a carnivore living in the Salonga Game and Forest Reserve which is close to the village of Bokela where the highest infection rate was observed.

Peel and Chardome (1946 and 1947) have precisely described from the chimpanzee in the Zaïre three new species of *Dipetalonema* (*D. vanhoofi*, *D. binucleata* and *D. rodhaini*). The microfilaria of *D. rodhaini*, found in the skin is characterized by its great length (318 to 347 μ) and the unusual position of the various organs. The microfilaria of *D. binucleata* is characterized by the presence of two paired nuclei in the tip of the tail. This microfilaria has also been found only in the skin. The microfilaria of the third species, *D. vanhoofi*, resembles closely that of *D. perstans* but is a little smaller. The average length is 154.34 μ in slides stained by the haematoxylin « wet method » and 182.82 μ in those stained by the haematoxylin « dry method » (mean for 25 microfilariae in each series). The lengths of the microfilariae of *D. perstans* from man collected in the same area and stained by the same methods were 171.68 μ and 196.46 μ respectively (Peel and Chardome, 1946).

D. semiclarum sp. nov. differs from *D. vanhoofi* in several important characters. First is the length of the body which is significantly greater than in *D. perstans* (measurements of specimens present in the same thick drop and therefore dried and stained identically). The microfilaria of *D. vanhoofi* is on the contrary, shorter than *D. perstans*. Another character is the structure of the excretory pore. In *D. vanhoofi* this pore is followed by a large excretory cell which in its turn is followed by a large clear spot. This characteristic structure is clearly shown on plate XVIII in the paper of Peel and Chardome. A third difference is the absence in *D. vanhoofi* of a long clear band in the posterior half of the body.

D. semiclarum differs from *Dipetalonema gorillae* Van den Berghe and Chardome (1949), described from *Gorilla gorilla*, in Kivu, Zaïre, by the greater length and width. In *D. gorilla* these measurements are 170.8 μ (range 158.9 - 175.5 μ) and 3.4 μ respectively. The authors noticed that these

TABLE 3

Distance from the anterior extremity of the organs expressed both in microns and as a percentage of the total body length, in *Dipetalonema semicilarum* sp. nov.

	Mean (in μ)	Range (in μ)	S. D.	S. E.	C. V.	%	
						mean	range
50 microfilariae measured in thick drops (by night)							
AE - N1	4.01	2.0- 7.0	0.91	0.12	22.74	2.02	1.0- 3.2
AE - NR	44.38	39.0- 50.0	2.46	0.34	5.53	22.47	19.6-25.0
AE - EP	60.76	55.0- 68.0	3.03	0.42	4.99	30.77	27.5-33.5
AE - BCB	108.22	96.0-120.0	5.80	0.81	5.35	54.77	49.2-58.3
AE - ECB	139.22	126.0-156.0	7.02	0.99	5.04	70.46	65.8-73.5
AE - A	164.62	150.0-181.0	8.02	1.13	4.87	83.32	77.3-86.4
Total length	197.58	184.0-220.0	8.78	1.24	4.44	—	—
30 microfilariae measured in dermic films (by day)							
AE - N1	4.78	3.0- 6.5	0.92	0.17	19.44	2.16	1.3- 2.8
AE - NR	51.93	48.0- 62.0	3.08	0.56	5.93	23.50	21.4-28.1
AE - EP	70.53	64.0- 76.0	3.08	0.56	4.36	31.91	30.3-34.5
AE - BCB	120.23	110.0-133.0	6.58	1.20	5.47	54.40	45.6-58.7
AE - ECB	155.66	142.0-171.0	6.95	1.26	4.47	70.41	67.5-73.0
AE - A	184.90	168.0-205.0	8.06	1.47	4.36	83.64	80.2-86.2
Total length	221.10	203.0-242.0	9.42	1.72	4.26	—	—

AE - N1, anterior extremity to first nucleus (cephalic space length); AE - NR, anterior extremity to nerve ring; AE - EP, anterior extremity to excretory pore; AE - BCB, anterior extremity to beginning of clear band; AE - ECB, anterior extremity to end of clear band; AE - A, anterior extremity to anal spot; S. E., standard error; S. D., standard deviation; C. V., coefficient of variation.

microfilariae were smaller than their specimens of *D. perstans* and of *D. vanhoofi* which had been dried and stained in the same condition. Other differential characters are in *D. semiclarum* the presence of a long clear band in the posterior half of the body and the much more anterior position of the anal spot (83.3 - 83.6 per cent instead of 92 per cent for *D. gorillae*).

Another species described from the gorilla is *Microfilaria leopoldi* Van den Berghe, Chardome and Peel, 1964. This species is characterized by the presence in the posterior end of a unique bunch of six closely packed terminal nuclei arranged in three pairs, the equal width of both ends, and the greater thickness of the body (width 11 μ).

D. semiclarum resembles the microfilaria of *Dipetalonema digitatum* (Chandler, 1929) described from gibbons in Asia. This species has been found again in *Macacus speciosa* from Bengal and redescribed by Webber and Hawkins (1955). The two species have in common the curious anatomical feature that we have described above, the long clear region situated in the posterior half of the body. In this area the nuclei are fewer in number and more widely spaced than in the rest of the nuclear column. The microfilaria of *D. digitatum* is however distinct from our species by the smaller width of the body (only 3 μ wide), the different structure of the tail (last nucleus thicker and often with a slight constriction just in front of this nucleus; single file of last nuclei shorter), the more posterior situation of the nervous ring (24 - 25.6 per cent), of the excretory pore (35.6 - 40.5 per cent), the anal pore (87.7 per cent) and the more anterior position of the clear zone (49.3 to 49.8 per cent).

Several other species of unsheathed microfilariae of the blood have been described from African monkeys, and some of them resemble *D. semiclarum* to some extent, but apparently none of them has this long clear area in the posterior half of the body that seems to be characteristic of our species and of *D. digitatum*. The two species that resemble our species the most closely are the following :

— *Microfilaria cercopithecii* Leger, 1922. From *Cercopithecus nictitans büttikoferi*, in French Guinea. Length 180-210 μ . Unsheathed. Tail long and strongly attenuated. Webber (1955) surmized that this species belongs probably to the genus *Dirofilaria*.

— *Microfilaria guerezae* Archetti, 1947. From *Colobus polykomos polyurus*, Abyssinia. Length 216.5 μ . Width 5.2 μ . Anal pore at 87.3 per cent, first nucleus at 2.9 per cent from the head.

By the shape of the tail, short with blunt tip and with nuclei reaching the tip or nearly so, and the absence of a sheath, the new microfilaria that we describe here should be placed in the genus *Dipetalonema*. It does not correspond exactly with any known species in that genus or in other genera and we believe that it is a new species.

Types : Typical slide is deposited in the Institut de Médecine tropicale Prince Léopold, Antwerp, Belgium. Paratypical slides in the Museum d'Histoire naturelle, Paris (Service of Prof. A. Chabaud), the School of Tropical Medicine, London, the School of Tropical Medicine, Liverpool, the Musée royal de l'Afrique centrale, Tervuren and in the collection of the author.

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Résumé — *Dipetalonema semiclarum* sp. nov. dans le sang de l'homme en République du Zaïre (Nematoda : Filarioidea).

L'auteur décrit une nouvelle microfilaire, *Dipetalonema semiclarum* sp. nov., découverte dans le sang de l'homme dans la Province de l'Équateur, République du Zaïre. Cette microfilaire fut rencontrée chez 52 habitants de trois villages. A Bokela, le village le plus fortement infesté, elle fut découverte chez 49 p. cent. de la population adulte. Le nombre maximum de microfilaries observé dans une goutte épaisse était de 210. Cette microfilaire est dépourvue de gaine, elle est subpériodique avec un petit sommet diurne. Elle mesure 221μ sur 5μ (moyennes mesurées dans le sang des frottis dermiques) ou 198μ sur $5,2 \mu$ (moyennes dans les gouttes épaisses). Son aspect général rappelle *D. perstans* mais elle est plus large et dans sa moitié postérieure il y a une bande claire contenant un petit nombre de noyaux groupés plus ou moins régulièrement.

Samenvatting — *Dipetalonema semiclarum* sp. nov. in het bloed van de mens in de Republiek Zaïre (Nematoda : Filarioidea).

Een nieuwe microfilaria *Dipetalonema semiclarum* sp. nov. wordt beschreven. Ze werd ontdekt in het bloed van de mens in de Evenaars Provincie, Republiek Zaïre. De microfilaria werd aangetroffen bij 52 inwoners van 3 verschillende dorpen. In Bokela, het meest geïnfecteerde dorp, werd ze gevonden bij 49 ten honderd van de volwassen bevolking. Het grootste aantal microfilaria bedroeg 210 per dikke druppel. Deze microfilaria is niet voorzien van een schede en is sub-periodiek met een lichte piek gedurende de dag. Ze meet 221μ bij 5μ (gemiddelden gemeten in het bloed van huidafnamen). Haar algemeen uitzicht lijkt op *D. perstans* doch ze is breder en in de achterste helft is er een lange klare band die een aantal grote of min of meer gegroepede kernen bevat.

A. Fain : Professor of Parasitology, Prince Leopold Institute of Tropical Medicine, Antwerp and Catholic University of Louvain, Belgium. Head of the Department of Medical Zoology, Prince Leopold Institute of Tropical Medicine, Nationaalestraat 155, B-2000 Antwerpen, Belgium.
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REFERENCES

- Archetti, I. (1947) : Descrizione di alcune nuove microfilarie di vertebrati africani. *Riv. Parassit.* **8**, 4, 175-190.
- Chabaud, A. G. (1952) : Le genre *Dipetalonema* Diesing. Essai de Classification. *Ann. Parasit. hum. comp.* **27**, nos 1-3, Addendum p. 284-285.
- Chardome, M. and Peel, E. (1951) : Une nouvelle filaire chez l'homme au Congo belge *Tetrapetalonema berghei* n. sp. *Ann. Soc. belge Méd. trop.* **31**, 571-580, 4 pl.
- Deschiens, R. and Pfister, R. (1954) : Sur une microfilaire observée dans le sang de l'homme en Haute Volta. *Bull. Soc. Pathol. Exot.* **47**, 278-282, pl. V.
- Fain, A., Wéry, M. and Tilkin, J. (1969) : Recherches sur les Filarioses humaines dans la région de la Cuvette Centrale (République Démocratique du Congo). *Ann. Soc. belge Méd. trop.* **49**, 6, 629-648.
- Fain, A., Elsen, P., Wéry, M. and Maertens, K. (1973) : Les filarioses humaines au Mayumbe (République du Zaïre). Description d'une méthode d'évaluation de la densité microfilarienne. *Ann. Soc. belge Méd. trop.*, **54**, 1, 5-34.
- Fullerborn, F. (1929) : Filarioses des Menschen. In Kollé and Wassermann's « Handbuch der pathogenen Mikroorganismen » 3rd ed. 6. Jena, Fischer pp. 1043-1224, pl. I-III.
- Leger, M. (1922) : Microfilaire sanguicole nouvelle du *Cercopithecus butikoferi*. *Compte Rendu des Séances de la Société de Biologie. Paris* **86**, 835.
- Peel, E. and Chardome, M. (1946) : Sur des filaridés de Chimpanzés « *Pan paniscus* » et « *Pan satyrus* » au Congo belge. *Ann. Soc. belge Méd. trop.* **26**, 117-156, pl. I-XXIII.
- Peel, E. and Chardome, M. (1947) : Note complémentaire sur les filaridés des Chimpanzés *Pan paniscus* et *Pan satyrus* au Congo belge. *Ann. Soc. belge Méd. trop.* **27**, n° 2, 241-250, pl. I-VIII.
- Schacher, J. F. (1969) : Intraspecific variation in microfilariae, with description of *Wuchereria lewisi* sp. nov. (Nematoda, Filarioidea) from man in Brazil. *Ann. Trop. Med. Parasit.* **63**, 3, 341-351.

- Van den Berghe, L. and Chardome, M. (1949) : Une microfilaire du gorille, *Microfilaria gorillae*. Ann. Soc. belge Méd. trop. **29**, 495-499.
- Van den Berghe, L., Chardome, M. and Peel, E. (1964) : The filarial parasites of the eastern gorilla in Congo. JI Helmit. **38** (3/4), 349-368.
- Webber, W. A. F. (1955) : The filarial parasites of Primates, a review. I. *Dirofilaria* and *Dipetalonema*. Annals Trop. Med. Parasit. **49**, 2, 123-141.
- Webber, W. A. F. (1955) : The filarial parasites of primates, a review. II. *Loa*, *Protofilaria* and *Parlitomosa*, with notes on incompletely identified adult and larval forms. Annals Trop. Med. Parasit. **49**, 3, 235-249.
- Webber, W. A. F. and Hawking, F. (1955) : The filarial worms *Dipetalonema digitatum* and *D. gracile* in monkeys. *Parasitology* **45**, 3/4, 401-408.
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