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SYMPOSIUM

Dynamics of Ecology of Free-Living Protozoa

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Editor is responsible for not correcting
some printer's errors. I corrected only
those which could be misleading.

W. Foissner

INTRODUCTION

The papers presented at the symposium S-12 on occasion of the VIII International Congress of Protozoology (July 10–17, 1989, Tsukuba Japan) were closely related to those read at a symposium of the VII Congress [1]. The contributions emphasized the importance of protozoa in the biotic cycles of fresh water, terrestrial and marine ecosystems and as ecological indicators.

Symposium S-15 (Marine Protozooplankton, chaired by Dr. F. Rassoulzadegan) and the plenary lecture P-6 by Dr. Tom Fenchel, who highlighted the role of protozoa in nature in terms of physiological constraints of protozoan organization, were related to S-12. Fenchel's recently published book "Ecology of Protozoa" is highly recommened to all who are interested in modern protozoan ecology and its relationship to general ecology [2].

Fresh water

Methodological problems were addressed by all speakers in this symposium, especially by Drs. J. R. Pratt and J. Cairns, Jr. (The Pennsylvania State University, School of Forest Resources, University Park, PA 16801 and Virginia Polytechnic Institute and State University, Biology Department and University Center for Environmental and Hazardous Materials Studies, Blacksburg, VA 24061, USA) in their paper "Methods for studying dynamics of fresh water protozoan communities under natural and anthropogenic stress".

Most fresh water protozoa and many marine forms are adapted to clinging, crawling, and gliding on natural substrate. Sampling must be directed at these surfaces if the protozoa are to be adequately studied, especially when environmental impact is to be evaluated because the discharge of wastes almost always occurs into the shallow zones of streams, lakes, and estuaries.

The major problem faced in sampling natural substrate is the difficulty in knowing the state of devolopment of the community. It is usually impossible to know when the last time a disturbance occurred. Pratt *et al.* [3] compared the diversity of protozoa on natural and artificial substrata in the Flint River (Georgia, USA) basin both in the river proper and in an impoundment. Artificial substrata produced collections with significantly higher population sizes than natural substrata, making resolution of extant species and community differences much easier (Table 1).

The use of artificial substrata simplifies studies of community dynamics because identical habitat patches can be sampled. Pratt's group has used polyurethane foam units (PFU's)—a three dimensional substratum—but other investigators have used glass slides, petri dishes, and even natural leaves and rocks. By controlling the exposure period of the substratum, the time-dependent processes structuring protozoan communities can be discerned.

Biogeographic theory predicts that communities will increase in diversity. The saturation value or equilibrium species number and the colonization rate are determined by biotic and abiotic condi-

TABLE 1. Numbers of protozoan species collected from natural and artificical substrata, Flint River (after Pratt *et al.* 1987)

Site	Natural		Artificial	
	Mean	S. D.	Mean	S. D.
RIVER				
Spring	41	17	99	6
Summer	46	8	161	11
Fall	49	13	134	17
LAKE				
Spring	47	16	101	17
Summer	51	14	147	14
Fall	44	16	140	13

S. D. = standard deviation.

TABLE 2. Chemical and biological parameters at 16 stations in the water system of Changde City (China)

Group Level of pollution Stations	I Very heavy 9	II Heavy 10, 12, 15, 16	III Diffuse 6, 7, 8, 11, 13, 14	IV Clean 2, 3, 4, 5	V Yuan Jiang River 1
Chemical combined pollution index	13	24.2	3.8	1.2	0.6
Heterotrophic index	208	137.7	78.3	58	—
Diversity index	2.1	3.5	3.9	3.5	3.1
Equilibrium species number	18.5	43.8	48.8	46.3	54.8
Colonization rate constant	1.6	1.6	1.2	0.7	0.8
Time (days) to get 90% of equilibrium species number	1.6	2.5	2.4	4	8.5

tions of habitat suitability. The different communities sampled through time differ considerably in both the numbers and kinds of extant species. For example, two communities each containing 50 species at equilibrium might attain this equilibrium by very different paths. This was impressively demonstrated by Dr. Y.-F. Shen (Academia Sinica, Institute of Hydrobiology, Wuhan Hubei, P. R. China) in her lecture "Biomonitoring by using protozoan communities". Dr. Shen applied the PFU method to Chinese city waters (Table 2) and showed that biotic parameters (heterotrophic index, colonization rate etc.; see also [4] were closely correlated with the chemical combined pollution index.

Nutrient inputs increase the rate of production and the rate of protozoan colonization. For example, in studies of two rivers feeding an impoundment, protozoan colonization of artificial substrate was stimulated in the river receiving excess nutrients (comp. Table 2).

Toxic materials inhibit protozoan colonization. This effect has been repeatedly shown by Dr. Shen and others. Pratt and Cairns studied a hard water stream in Pennsylvania which received sewage water that contained chlorine, ammonia, and chloramines which are very toxic. After 7 days, protozoa at upstream reference stations showed healthy communities but downstream communities were seriously affected, and even during flood stage the impact of the effluent was observable as reduced numbers of protozoan species (Fig. 1).

Artificial substrata provide opportunities to bring natural assemblages of protozoa and related biocoenoses into the laboratory for study. Effects of human influences such as toxic materials can be examined in microcosms. Sometimes the results are remarkable demonstrations of important ecological phenomena.

Pratt *et al.* [5] examined the effects of the

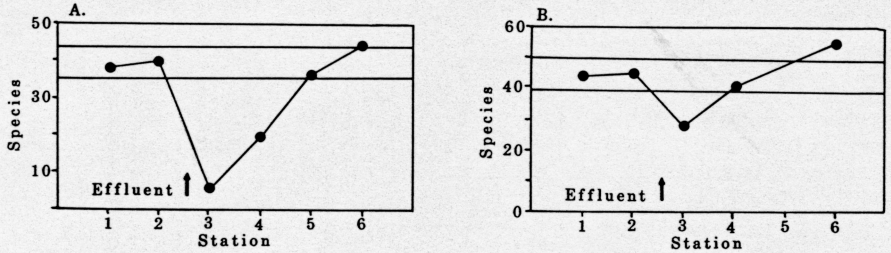

FIG. 1. Protozoan species numbers on artificial substrata above and below a toxic municipal water source. A. normal flow, B. flood stage.

herbicide atrazine on protozoan communities on artificial substrata in microcosms receiving a continuous input of atrazine-endowed water and found that high dosages severely affected algae and protozoa, but low dosages led to increase in the numbers of protozoan species (Fig. 2). When algae are killed by toxic chemicals an important portion of protozoan food organisms is killed. Low levels of toxic materials only partially affect algae while upsetting the normal balance of the community. Surprisingly, this can result in more - not fewer - species, with striking changes in species composition. Similar effects have been frequently reported [6].

Dr. Shen achived important results using PFU epicenters in a flow-through microcosm system. She successfully applied this test-system in estimating the median lethal concentration and the maximal acceptable toxic concentration of various chemicals on individual ciliate species and on protozoan communities.

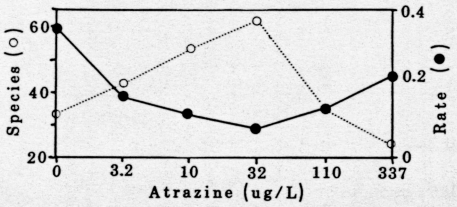

FIG. 2. Protozoan colonization dynamics in atrazine-amended microcosms. Colonization rate (G) based on fit to model $S_t = S_{eq}(1-EXP(-GT))$ (after Pratt *et al.* [5]).

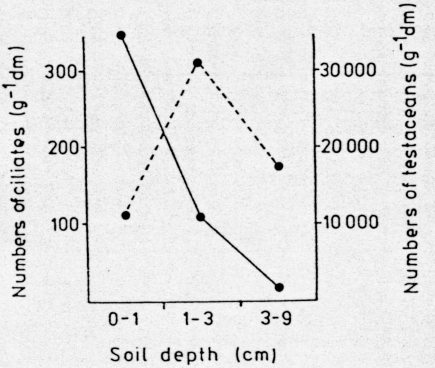

FIG. 3. Spatial separation of active ciliates (——) and testate amoebae (----) in a spruce forest (from Petz & Foissner [10]).

Soil

Dr. W. Foissner (Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria) reported on "Ciliatostasis: a new approach in soil protozoology". The rather restricted taxonomic knowledge of many soil protozoologists and the almost exclusive use of Singh's culture method and its modifications in evaluating the abundance of terrestrial ciliates resulted in a very distorted picture of that what actually happens in the soil. This is evident from investigations performed by Foissner's group during the last 10 years. They used a direct counting technique and a simple but effective culture method ("non-flooded petri dish method"; [7]) to estimate the number and variety of species present. In the course of these investigation it became clear that active ciliates occur in very low number (0–100 individuals/g dry mass of soil) in all cultivated and naturally evolved soils, whereas they are often fairly abundant (500–3000 individuals/g) in fresh litters and other non-evolved soils (Table 3). No such differences could be ascertained in the number of species.

The concept of ciliatostasis was introduced in order to describe and to explain, at least partially, these startling results [7]. The term "ciliatostasis" refers to the phenomenon that excystment and growth of ciliates in naturally evolved and cultivated soils is far lower than might be expected under similar conditions of temperature, moisture, pH, etc. *in vitro*. The term is derived from "fungistasis" and is restricted to the designation of the phenomenon itself and should not carry any connotations as to causality.

Ciliatostasis can be relieved or even annulled by additing energy-containing nutrients to the soil (e.g. glucose or plant residues, which is exploited by the non-flooded petri dish method). This suggests that food is an important factor. On the other hand, there is certainly abundant food in the upper, densely rooted layers of meadows and pastures and in the fermentation and humus layers of forests where few or no active ciliates are present (Fig. 3). This suggests that food is most likely superimposed on, and secondary to, a more

TABLE 3. Abundance ($\bar{x}$) of active ciliates in frech and in air-dried soils six days after rewetting (from Foissner 1989)

Site[1]	Active ciliates/g dry mass of soil[2]		
	Fresh soil	Air-dried soil	Increase %(x 10^3)
Cultivated field (n=8)	1	379	37.9
Meadow (n=8)	3	520	17.3
Coniferous litter 1 (n=1)	319	11430	3.6
Coniferous litter 2 (n=1)	468	17390	3.7
Beech-wood litter (n=1)	3326	104340	3.1

[1] Investigated layers: field 5–15 cm, meadow 0–5 cm, forests 0–2/3 cm.
[2] Methods see [7] and [9]

pervasive ciliatostasis derived from as yet unknown inhibitory substances present mainly in evolved soils. This is supported by fertilization experiments showing an increased abundance of active ciliates only after removal of the evolved top soil [8, 9].

Soil ciliatostasis and soil microbiostasis in general may be a powerful means of niche separation and of maintaining the equilibrium between the different soil organisms. It results, for instance, in a fairly distinct separation of ciliates and testaceans in spruce forests (Fig. 3). The ciliates are most abundant in fresh litter, whereas the testaceans are most abundant in the fermentation and the humus layer [10]. This study and other published data indicate that testaceans are not or less subjected to soil protistostasis than ciliates, zooflagellates, and naked amoebae.

Little is known about the causes of the ciliatostasis. It is, however, evident that certain sustances which inhibit the growth of ciliates accumulate in evolved soils. Some experiments showed that these substances are probably produced by the activity of microorganisms. The inhibitory material is partially volatile and water-soluble (Table 4, 5).

The fairly high number of active ciliates present in fresh litters suggest that ciliates play an important role in the decomposer cycle and in the initial stages of soil development.

Activated sludge

Soil protozoa have long been thought to be widespread in activated sludge. Foissner [7] disproved this proposition, showing it to be caused by misidentification of species and the misunderstand-

TABLE 4. Abundance (arithmetic mean and standard deviation) of ciliates in fresh, washed and air-dried soils (from Petz & Foissner 1989)

Site	Soil depth (cm)	Ciliates/g dry mass of soil		
		Fresh soil	Washed soil[1]	Air-dried soil[2]
Cushion plant site (n=8)	0– 5	11 (±14.1)	30*[3] (±31)	1570 (±1910)
Alpine mat (n=10)	0–10	2 (± 3.2)	49** (±42)	134 (± 90)

[1] 0.2 g soil was several times washed with water, put into a small glass tube, which was sealed by a filter (0.25 μm meshes) on its lower end, and 14 days exposed at the same site where it has been sampled. Then the abundance of the ciliates was estimated with the direct method of Lüftenegger [9] and compared with the abundance occurring at the same time in the neighboured fresh soil.
[2] Culture method according to Buitkamp (1979).
[3] Different at $0.1<p<0.2$ (*) and $p<0.005$ (**) from the fresh soil and at $p<0.01$ (**) from air-dried soil with the U-test of Mann-Whitney.

TABLE 5. Effect of soil vapour on excystment of the soil ciliate *Oxytricha granulifera* (from Foissner 1989)

Treatment[1)]	% Excystment after 48 hr	Number of experiments
Soil vapour	46	24
Control	71	24

[1)] Two cysts each were transfered to small glass vessels containing an appropriate culture medium. The vessels were placed in some large petri dishes containing an undistorted upper (0–3 cm) soil layer of a meadow. The covers of the petri dishes were slightly raised to enable relatively unobstracted aeration during the experiment. Controls, identically treated except that the petri dishes were lined with moistened filter paper instead of soil, were set up.

ing of the ecological key factors determining protozoan communities in soil and sewage. The ecological aspects of "Protozoa in activated sludge" were excellently reviewed by Dr. P. Madoni (University of Parma, Institute of Ecology, I-43100 Parma, Italy) in the present symposium.

Biological sewage treatment plants can be regarded as artificial ecosystems subject to extreme conditions. As in every other biological system, the biocoenosis of an activated sludge plant has a structure (components and factors) and dynamics (in time and space) Regarding the biotic components, it is well-known that activated sludge develops specific communities of protozoa which are sustained by copious production of bacteria. Heterotrophic flagellates, sarcodines and ciliates, as well as various small metazoans like nematodes and rotifers occur, but cilliates have attracted the most attention.

Ciliated protozoa are numerous in all types of biological treatment systems; they are commonly found in densities of about 10000 cells per ml of activated sludge [11]. Counts indicate that protozoans make up approximately 5% of the dry weight of the suspended solids. Of the 230 species of protozoans observed in activated sludge, 160 are ciliates, less than half of which were found frequently [12, 13]. The ciliates of activated sludge can be subdivided into three groups on the basis of their behaviour: i) free-swimming in the liquid phase, remaining evenly dispersed in the sedimentation tank (e.g. *Litonotus* spp., *Colpoda* spp.); ii) crawling but free forms, inhabiting surface of sludge flocs (e.g. *Aspidisca cicada, Trochilia minuta, Euplotes* spp., *Chilodonella uncinata, Thigmogaster oppositevacuolatus*); iii) attached and strictly associated with the sludge flocs, thus precipitating during sedimentation (e.g. *Vorticella* spp., *Opercularia* spp., *Epistylis* spp., suctorians). The peritrich ciliates which filter out suspended bacteria are considered to be the most important group of protozoan in activated sludge because their activity reduce the turbitity of the effluent [14]. In contrast, peritrichs are rare in both numbers and species in soil [7]. The crawling forms, such as *Aspidisca* and *Chilodonella,* scrape bacteria from the furface of the flocs und thus influence their shape which in turn influences the sedimentation capacity of the sludge. Ciliates, moreover, remove the majority of fecal bacteria such as *Escherichia coli* from sewage [15].

In the aeration tank of activated sludge systems a true trophic web is established (Fig. 4). The growth of heterotrophic bacteria depends on quality and quantity of dissolved organic matter

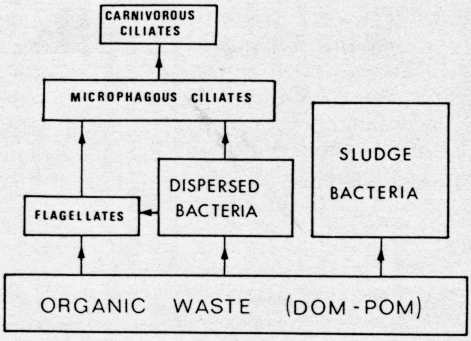

FIG. 4. Trophic web in the activated sludge of sewage treatment plants (from Madoni [13]).

(DOM). For predators, on the other hand, growth depends on the available prey. Dispersed bacteria are thus food of heterotrophic flagellates and bacterivorous ciliates which in turn become the prey of carnivorous organisms. Competition and predation create oscillations and successions of populations until dynamic stability is achieved. This is strictly dependent on plant type and management. By considering the activated sludge process as a chemostat [16] simulated the dynamics of a similar culture system using both experimental and theoretical model.

Results from recent studies on the modalities of colonization and of population succession in activated sludge demonstrated the determining effect of environmental conditions in the aeration tank on the ciliate community established [17, 18]. Species entering through sewage do not seem to play an important role in determining the structure of the community or the colonization of the sludge floc. The plant starting phase is characterized by the presence of species typical of raw sewage (Fig. 5). These "pioneer species" are represented chiefly by free-swimming bacteriovorous ciliates (above all hymenostomes) and flagellates, and are thus not linked to the presence of sludge; and

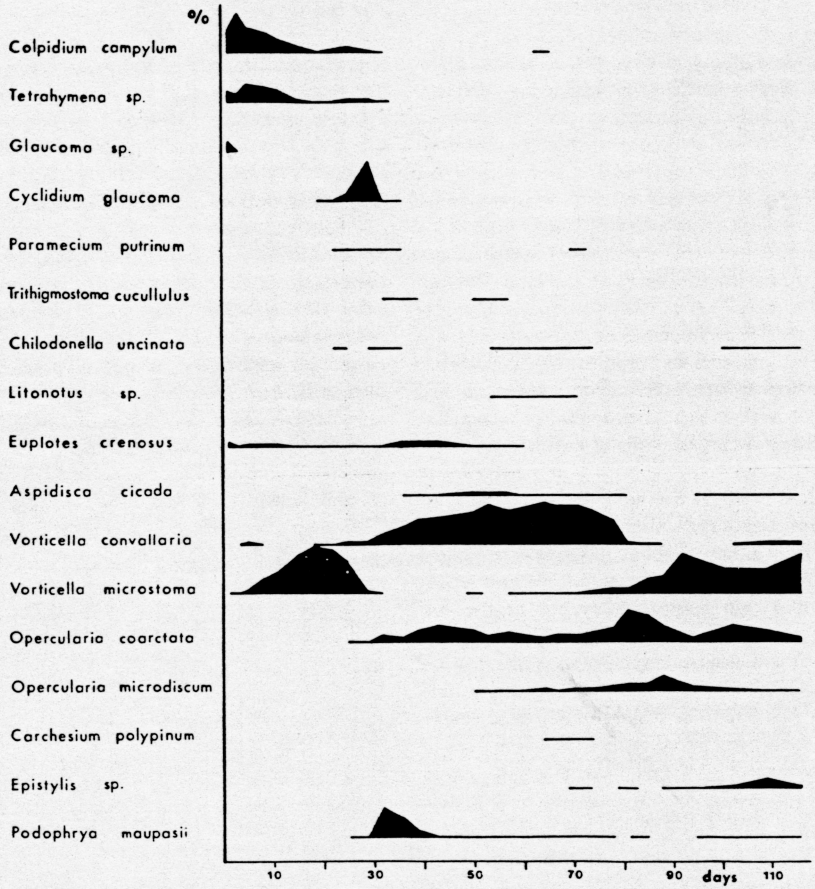

FIG. 5. Succession of ciliates during the colonization of an activated sludge plant (from Madoni&Antonietti [18]).

TABLE 6. Plant performance indicated by particular dominant groups of protozoa

Dominant group	Performance	Possible causes
Small flagellates	poor	poorly aerated sludge; overload; fermenting substances involved
Small free-swinning ciliates (<50 μm)	mediocre	too short sewage retention time; poorly aerated sludge
Large free-swimming ciliates (>50 μm)	mediocre	overloading
Crawling ciliates	good	
Attached and crawling ciliates	good	
Attached ciliates	decreasing	transitory phenomena (discontinuous load; recent sludge extraction; slow recycling time)
Small naked amoebae and flagellates	poor	Very high load, not easily degradable
Testate amoebae	—	low load

cannot be considered typical components of these environments. With the formation of the activated sludge, they compete with species better adapted to an aeration tank environment and rapidly decline in numbers. The steady-state phase is characterized by a ciliate community (attached and crawling forms) whose structure reflects the stable conditions of the aeration tank environment with its balance of organic loading and sludge produced, removed and recycled. The species structure of the microfauna is thus a diagnostic instrument serving both in integrating the parameters of plant performance and in predicting the effluent quality [19–21]. Some examples are shown in Table 6.

Aquaculture

The nutrient-rich effluent of activated sewage plants is frequently used to rear various freshwater fish (e.g. carp) in aquacultures. Traditionally, protozoologists are engaged in this process only as parasitologists. Dr. Maeda (University of Tokyo, Ocean Research Institute), however, reported on a new aspect, namely "The control of maricultures using bacteria and protozoa".

Almost the same numbers of bacteria (about 10^5–10^6 cells/ml) are found in the open, non-eutrophic oceans, in the eutrophic coastal sea areas and in maricultures. This surprising observation is explained by the much higher abundance of protozoa in eutrophic waters which graze on bacteria, keeping their number down to a fairly low and constant level. Maeda's group isolated a specific bacterial strains, which, when added to the maricultures, are fed on by prawns and repress the growth of pathogenic bacteria, especially *Vibrio* spp. and even fungi.

Most mariculturists agree that the growth of prawns is promoted when the diatoms used as food are in the post-logarithmic growth phase. This is ascribed to food-attached bacteria and protozoa which are few during the exponential growth phase of the algae. The suitability of ciliates as food organisms for prawn larvae has been tested with an undetermined species isolated from a prawn pond and with *Strobilidium sulcatum*. This species rendered a higher larval survival and moulting rate than did other ciliate *S. sulcatum*. But, the bacterial strains which supported the growth of *S. sulcatum* fatally suppressed the activity of the prawn larvae. Dr. Maeda's group was, however, recently successful in isolating a bacterial strain which promoted both the growth of *S. sulcatum* and shrimp larvae.

The data in this presentation suggest that it is possible to control maricultures via specific bacteria and protozoa. In addition presumably, the production of fish and crustaceans can be substan-

tially increased once we succeed in establishing a food chain from bacteria and protozoa to fish and prawn larvae.

Summary and conclusions

It is becoming more and more apparent that protozoa play an important role in the microbial food webs of running and stagnant fresh waters, in soil and in the open sea [2, 7, 22–24]; see also F. Rassoulzadegan's report in this volume). Fortunately, this is being increasingly acknowledged even by general ecologists.

In fresh water, future studies need to examine the link between community studies of environmental impact and the responses of particular populations, especially indicator species. Indicators of toxic pollution are needed—if they can be found.

Rather less progress is evident in terms of soil protozoology. More accurately designed field studies and improved methods are necessary to evaluate the contribution of protozoa to the energy budget of terrestrial ecosystems. Many of the modern microcosm experiments [25] neglect the fact that an air-dried and sieved and thus structurally distorted soil is substantially different from a field soil with an ordered sequence of layers and physico-chemical properties (Table 3).

Similarly, protozoa of activated sludge are a rather neglected field in recent times. Future studies should emphasize the important practical aspects and investigate the quantitative relationships in more detail, for which a standardized technique is now available [11].

Protozoologists should certainly become more engaged in aquaculture. It ought to be possible to establish and control a food chain from bacteria and protozoa to fish and shrimp larvae. This would increase artificial food compound efficiency and maintain aquacultures in a more healthy condition, resulting in increased fish and shrimp production.

Identifying protozoa is still a major problem in most ecological studies. This will, however, be solved, at least partially, in the near future. Updated keys to species are in preparation (see "Protozoenfauna" published by the G. Fischer-Verlag; first volume treating suctorians and mobiline peritrichs has already appeared [26, 27]. Foissner's group is preparing an atlas of 400 indicator ciliates [28, 29].

Taxonomists must provide ecologists with better keys. Ecologists must provide taxonomists with better identifications and distribution records. Much valuable information on species distribution is being lost because of the difficulties in identifying species. Many protozoan ecologists refuse to publish extensive species lists because they have neither time nor resources to use cytological staining to confirm many identifications.

We need to know much more about the annual patterns of appearance and disappearance of protozoa and their productivity relationships. Our knowledge of protozoa on continents other than North America and Europe is improving (see for instance [30, 31] valuable contributions to the ciliates of Africa and Australia) but there is still far too little exchange of information (probably because of inadequate species identifications) among protozoologists in Africa, Asia, Australia, and South America.

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