CRC

ECOLOGICAL RELATIONSHIPS and EVOLUTION of the RICKETTSIAE

Volume II

Nyven J. Marchette

CRC PRESS

Ecological Relationships and Evolution of the Rickettsiae

Volume II

Author

Nyven J. Marchette, Ph.D.
Professor of Tropical Medicine and Public Health
The John A. Burns School of Medicine
University of Hawaii
Honolulu, Hawaii

With contributions by

David Stiller, Ph.D.
Hemoparasitic Diseases Research Unit
Hemoparasite Laboratory
Agriculture Research Service
U.S. Department of Agriculture
Washington State University
Pullman, Washington

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Marchette, Nyven J., 1928–
Ecological relationships and evolution of the rickettsiae.

Bibliography: p.
Includes index.
1. Rickettsia. 2. Microbial ecology.
3. Rickettsia—Evolution. I. Stiller, David, 1931– . II. Title.
QR353.M37 576'.62 81-18005
ISBN 0-8493-6125-7 (v. 1) AACR2
ISBN 0-8493-6126-5 (v. 2)

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-6125-7 (Volume 1)
International Standard Book Number 0-8493-6126-5 (Volume 2)

Library of Congress Card Number 81-18005
Printed in the United States

PREFACE

"What song the Syrens sang, or what name Achilles assumed when he hid himself among women, although puzzling questions, are not beyond all conjecture." In these words Sir Thomas Browne sets no limit to speculation, but it is generally admitted that speculation is idle if it is useless. The present review of rickettsial ecology and evolution assumes that speculation should be carried as far as is necessary to form a working hypothesis, upon the framework of which both the investigator and the student may hang their ideas. Moreover, a detailed examination of the evolution and possible relationships of the pathogenic rickettsiae may provide the fundamental basis for a natural classification of the family Rickettsiaceae and perhaps even the order Rickettsiales.

The ecological relationships of the rickettsiae for the most part are well known or at least amenable to investigation, but many details relating directly to rickettsial evolution are missing or incompletely known and cannot be subjected to rigorous scientific analysis. Fortunately, however, rickettsiae are obligate parasites with complex life cycles dependent upon certain mammals and arthropods about which a great deal is known. Our knowledge of the present geographical distribution of vertebrates, acarines, and pathogenic rickettsiae is especially good, and immunological and biochemical techniques enable us to establish degrees of relationship between microorganisms with some confidence. Many aspects of the relationships of rickettsiae to their hosts are also well known. The paleontological evidence for the evolution of the mammalian hosts of ticks and their rickettsiae, though far from complete, is sufficient to satisfy most critics. The geological record of changes of the Earth over the past 100 million years or so is likewise well documented, if not complete in every detail. Ticks and mites are almost completely absent from the fossil record, but studies of the systematics and distribution of living forms provide valuable, if incomplete, information on acarine phylogeny. Judicious use of the considerable amount of material that is available relative to rickettsiae should enable one to speculate from a firm base as to the probable course of rickettsial evolution.

An axiom of science is that, in the study of natural phenomena, the simplest explanation that logically fits all the data available is likely to be closest to the truth. This assumes, of course, that all or most of the relevant information concerning the phenomenon under investigation is known, but this is not always the case, and seldom is it so in the study of evolution. Thus, it is essential not to construct too rigid a framework for the rickettsial evolutionary tree. The accumulation of new data may require its periodic pruning, with prudent cutting and grafting of branches here and there, to maintain its viability and preserve its natural symmetry.

In the present study, many fields and disciplines are explored for evidence pertinent to an evolutionary history of the rickettsiae. From these data may emerge a logical sequence of events occuring through geological and historical time, culminating in the rickettsial species living today. As you will see, in certain areas so little is known that the conclusions are scarcely more than educated guesses. Perhaps the most valuable contribution of this study will be in pointing out what is not known rather than in reviewing what is known of rickettsial evolution. If even a few students are stimulated to explore some of the many unsolved problems, this work will have served a useful purpose.

Nyven Marchette

ACKNOWLEDGMENTS

My thanks to all those who read and commented on portions of the manuscript while it was in preparation. I am especially grateful to Dr. Harry Hoogstraal and Dr. Robert Traub for invaluable critical comments. The views expressed in this work, however, are entirely my own, and I am wholly responsible for any errors of fact that may occur. Thanks also to Ms. Iris McCrea for verifying many of the references and to Ms. Francis Kramer, who did the bulk of the final typing.

THE AUTHOR

Nyven J. Marchette, Ph.D., is Professor of Tropical Medicine and Public Health at the University of Hawaii, John A. Burns School of Medicine, Honolulu, Hawaii.

He received a Bachelor of Science degree in general zoology from the University of California, Berkeley in 1950 and a Master of Science degree in Invertebrate Zoology from the same institution in 1953. The next two years were spent in the U.S. Army as an enlisted man assigned to the Biological Warfare testing facility at Dugway, Utah where he participated in ecological research in collaboration with a group from the University of Utah. Following discharge from the army, he stayed with the university group and received the Ph.D. degree in bacteriology from the University of Utah, Salt Lake City in 1960.

In 1960–1961 Dr. Marchette was appointed Research Bacteriologist at the University of Utah Ecological Research facility at Dugway Proving Ground, Utah. Between 1961 and 1964 he was a post doctoral fellow and then Assistant Research Microbiologist in the International Centers for Medical Research and Training Program at the University of California, San Francisco and the Institute for Medical Research in Kuala Lumpur, Malaysia. From 1965 to 1967, he moved to the Department of Microbiology in the new University of Malaya Medical School and established the University of California ICMRT Arbovirus Research Laboratory. He remained with the University of California until 1970 running the arbovirus research laboratory in San Francisco at the Hooper Foundation (still associated with the ICMRT).

In 1970, Dr. Marchette accepted a position as Associate Professor of Tropical Medicine and Public Health at the Department of Tropical Medicine and the School of Public Health at the University of Hawaii, Honolulu. From 1974 to the present his title has been Professor of Tropical Medicine and Public Health.

He is a member of The American Society for Microbiology and is currently president of the local Hawaii Branch. His other memberships include the American Society of Tropical Medicine and Hygiene, the American Association for the Advancement of Science, and Sigma Xi.

Dr. Marchette has been Investigator or Associate Investigator on numerous NIH and WHO grants studying viral pathogenesis (principally dengue viruses) and currently is Co-investigator on an NIH grant to determine the etiology of Kawasaki Disease.

In 1977–1978 he was awarded a Fogarty International Senior Fellowship at the John Curtin School of Medical Research, Australia National University, Canberra, Australia. Part of that year was spent in research on the material for this book.

Dr. Marchette's current research interests include the etiology and pathogenesis of viral and rickettsial infections, the ecology of *Ehrlichia* and related rickettsiae in Hawaii, and the epidemiology of virus diseases in Hawaii and the Pacific Basin.

Dr. Marchette has published over 50 articles in the field of Microbiology.

Ecological Relationships and Evolution of the Rickettsiae

Nyven J. Marchette and David Stiller

Volume I

Volume II

TABLE OF CONTENTS

Volume II

Chapter 7

RICKETTSIA TSUTSUGAMUSHI—AN ACARINE OFFSHOOT OR A SEPARATE LINE?

N. Marchette

TABLE OF CONTENTS

I. INTRODUCTION

Rickettsia tsutsugamushi (=*R. orientalis* in Japanese and older literature) is the only rickettsia or rickettsia-like organism known to be biologically associated with trombiculid mites. Its relationship to other acarineborne rickettsiae (the tickborne or spotted fever complex) is unclear. However, it replicates exclusively in the cytoplasm and does not infect the nucleus of the cell. Infection induces the production of antibody to the OX K strain of *Proteus vulgaris,* but not to the OX 19 or OX 2 strains which are agglutinated by antibody to insectborne and tickborne rickettsiae.

The disease in man caused by *R. tsutsugamushi* is known by a variety of names,[1,2] the most common being scrub typhus in Western and tsutsugamushi disease in Japanese literature. The disease, also called *Akadani-byo,* was recognized by the Japanese centuries ago as a specific disease entity, and they associated it, as the names imply, with mites (Kadani, Akamushi, or Tsutsugamushi).[1,3] A prodigious amount of work was done by the early Japanese workers, but it wasn't until after World War II that the ecology of tsutsugamushi fever was elucidated,[3] and species of *Apodemus, Microtus,* and other rodents and trombiculid mites were recognized as major carriers of the rickettsiae. Traub and Wisseman[2] discussed the confusing and inaccurate terminology used for this disease and concluded that chiggerborne typhus is most appropriate for the disease in man and chiggerborne rickettsiosis for the infection in general. Their terminology is accepted and used here (see Volume I, Chapter 2).

II. GEOGRAPHICAL AND HOST DISTRIBUTION

A. Geographical Distribution

A good deal of the research on chiggerborne rickettsiae and particularly chiggerborne rickettsioses has been done in Japan, Malaya (now West Malaysia), India, and Australia. The disease also occurs in Taiwan,[4–7] eastern China,[8] and Korea.[9] Once thought to be distributed primarily in Japan and the Indo-Malaysian-Australasian Region, *R. tsutsugamushi* is now known to occur in a variety of small mammals in high mountain valleys, mountain deserts, and semidesert areas of West Pakistan.[10] Other investigations have added two widely separated areas of Russia to the known distribution of *R. tsutsugamushi:* Tadzikistan in the southwest,[11,12] and the Primorye Region of southeast Siberia[12–14] (Tables 1 and 2). It has been isolated from man, rodents and marsupials,[54] and *Leptotrombidium deliense*[44] in Queensland, Australia. This chigger is abundant on forest and scrub mammals in the endemic areas and is circumstantially associated there with chiggerborne typhus in man.[55]

The limits of the geographical distribution of *R. tsutsugamushi* may not be known with great precision, but it is clear that it is indigenous to the Old World Tropics of Asia with extensions south to New Guinea and tropical or semitropical Australia and north to southern portions of the Palearctic. It has never been recovered in Africa, Europe, nor the New World, and there is no reason to believe that it has ever occurred west of Iran.[56] Reports of the possible occurrence of chiggerborne typhus in Africa and Arabia are based entirely on serological data. Freyche and Deutschman[57] reviewed the situation before 1950 and concluded that evidence for its existence in Africa was not clear. Later, reports were published on the discovery of complement-fixing and agglutinating antibody to *R. tsutsugamushi* in the sera of African rodents.[58,59] Also, Mount and Baranski[60] reported the presence of OX K agglutinins in human sera from a mountainous district in Yemen. However, the well-known complexity of serological reactions is no less true in this case. *Proteus* OX K agglutinins are especially poor indicators of chiggerborne rickettsiosis infections.[61–65] In the absence of a valid rickettsial isolation,

Table 1
TROMBICULID MITE HOSTS OF *RICKETTSIA TSUTSUGAMUSHI*[a]

Species	Locality	Isolations: Reported	Isolations: Suspected
Ascoschongastia audyi (Womersley)	Malaya	15[b]	
A. indica (Hirst)	China	8[c]	
Euschongastia ikaoensis Sasa et al.[d]	Japan	17, 18	
Eutrombicula wichmanni (Oudemans)	Philippines		19
Gahrliepia ligula Radford	India, Burma	20	
G. saduski Womersley	Japan	17	
Leptotrombidium akamushi (Brumpt)	Japan	3, 21	
L. arenicola (Traub)	Malaya	22, 23	
L. deliense (Walch)	Malaya	24, 25	
	Thailand	26	
	India, Burma	27	
	Philippines	19, 28	
	Indonesia	29	
	New Guinea	30[e]	
	Australia	32	
	Taiwan	33	
	China	8	
	West Pakistan		10
L. dihumerale (Traub and Nadchatram)[f]	West Pakistan		10
L. fletcheri (Womersley and Heaslip)	Malaya	24	
	Philippines		28
	Indonesia		34
	New Guinea	30,[g] 35	
	Taiwan		4[h]
L. gliricolens (Hirst)	West Pakistan		10
L. irregulare Traub and Nadchatram[f]	West Pakistan		10
L. jayewickremei (Womersley)	West Pakistan		10
L. kawamurai Fukuzumi and Obata	Japan	17, 36	
L. murotoensis Sasa and Kawashima	Japan	17	
L. orientalis Schluger	Siberia	36[a]	
L. pallidum (Nagayo et al.)	Japan	3,[i] 37	
	Korea	9	
	Siberia	36[a]	12[j]
L. palpale (Nagayo et al.)	Japan	17	
	Siberia	11, 36[a]	
L. pavlovskyi (Schluger)	Siberia	11, 36[a]	12,[j] 38
L. rupestre (Traub and Nadchatram)[f]	West Pakistan		10
L. scutellare (Nagayo et al.)	Japan	3,[i] 18, 37	
L. subintermedium (Jameson and Toshioka)	West Pakistan		10
L. tithwalense (Womersley)	West Pakistan		10
L. tosa (Sasa and Kawashima)	Japan	37	
Neotrombicula japonica (Tanaka)	Siberia	11, 36[a]	12,[j] 38
N. mitamurai (Sasa et al.)	Siberia	11, 36[a]	
N. pomeranzevi Schluger	Japan, Kuriles	17	39[k]

[a] This is not an exhaustive list of references to *R. tsutsugamushi* isolations from chiggers. Those given are generally the original sources or the ones in which definitive work is reported.

[b] Reported as *Euschongastia indica* (Hirst), but subsequently considered to be a new species, *Ascoschongastia audyi* (Womersley, 1952).[2]

[c] Reported as *Euschongastia indica* (Hirst). The authors also claim to have isolated *R. tsutsugamushi* from pools of *Acomatacarus* sp. and *Gahrliepia (Walchia)* sp. Confirmation of this work is needed. Shrai et al.[8a] demonstrated *R. tsutsugamushi* in *A. indica* by the fluorescein-tagged antibody technique.

Table 1 (continued)
TROMBICULID MITE HOSTS OF *RICKETTSIA TSUTSUGAMUSHI*[a]

[d]Kitaoka et al.[17] list this species as *Eutrombicula ikaoensis*, and Asanuma et al.[18] list it as *Cheladonia* (presumably a misspelling of *Chelodonta*) *ikaoensis*.

[e]The infected mites are reported as *Trombicula walchi* Womersley and Heaslip. This species is now considered synonymous with *L. deliense* (Walch), as is *T. vanderghinstei* Gunther.[31]

[f]These species, originally referred to as new species #1, #2, and #3,[10] are *L. dihumerale*, *L. irregulare*, and *L. rupestre*, respectively.[2]

[g]Reported as *Trombicula fletcheri*, now *Leptotrombidium fletcheri*. The isolations were made from pools of mites containing mostly this species, but in some instances other species were also included. All references to *L. akamushi* outside Japan are probably referrable to this species.[2]

[h]Monkeys exposed to a mite-infested area of Taiwan contracted a febrile disease similar to tsutsugamushi fever. Numerous *Trombicula akamushi* (probably *Leptotrombidium fletcheri*) were found attached to the monkey.

[i]Tamiya[3] cites references to the original work, most of which are in Japanese.

[j]Strains of *R. tsutsugamushi* were isolated from pools containing mixed species of trombiculids including *L. pallidum*, *L. pavlovskyi*, *N. japonica*, and *L. orientalis*. Since *L. pallidum* is known to be naturally infected in Japan, probably it is also naturally infected in the Primorsk region of Siberia, which resembles northern Japan. Pools containing only *L. pavlovskyi* and *N. japonica* also yielded *R. tsutsugamushi*. There is no way to determine which species contributed the rickettsiae to the pool.

[k]Reported as *D. pomeranzevi*, the *D.* probably referring to *Digenualeae*, a synonym of the subgenus *Neotrombicula*.[2]

one must remain skeptical that the data show the existence of *R. tsutsugamushi* in Africa and Arabia. Traub and Wisseman[2] considered it possible that chiggerborne rickettsiosis (not necessarily chiggerborne typhus) might occur in isolated areas (oases) in the Middle East and Africa, but thought it very unlikely that it has ever occurred in the New World.

It is important that we keep an open mind regarding the limits of *R. tsutsugamushi* distribution because the distribution of the human disease is not necessarily that of the agent that causes it. Among all rickettsial species known, *R. tsutsugamushi* is the most variable in terms of adaptability and virulence for laboratory animals. Pathogenicity for man appears to be equally variable,[66,67] but in general may be fairly constant for each locality. In some situations, strains may exist which are of such low virulence for laboratory animals that they are difficult to detect by the usual methods.

Rickettsia tsutsugamushi is a complex of serotypes which may be as diverse as those of the tickborne rickettsiae but not so widely distributed nor so well characterized; the number of distinct serotypes that exist is not known. The antigenic diversity of strains from the same and different regions has been repeatedly demonstrated by complement-fixation tests using soluble and partially purified antigens, serum neutralization, vaccination cross-protection, toxin neutralization, and immunofluorescence tests[68–80] (see also a review of earlier Japanese work by Tamiya[3]). The three classical serotypes are Karp, Gilliam, and Kato,[70] but there is some evidence for other intermediate and distinct serotypes.[17,71,81] The Karp strain was isolated from a patient in New Guinea,[82] Gilliam from a patient near the Assam-Burma border,[49] and Kato from a patient in Niigata Prefecture, Japan.[83]

Japanese strains all apparently belong to one or another of the three classical types. Strains isolated from man, rodents, and chiggers in the Primorye Territory, U.S.S.R. were all identified as belonging to serotype Gilliam[84] or heterogeneous Gilliam-Karp or Karp-Gilliam serotypes,[85] while strains in the southern Kurile Islands were all closely related to Karp.[86] In Tadjikstan, both Gilliam and Gilliam-Karp serotypes occur; in Armenian S.S.R., the Gilliam serotype prevails.[85] Elisberg et al.[71] isolated five new serotypes in addition to the three classical ones in Thailand. Immunofluorescence tests revealed a multiplicity of antigens shared among them. A study of 79 strains isolated

Table 2
VERTEBRATES REPORTED INFECTED WITH *RICKETTSIA TSUTSUGAMUSHI*

Species	Locality	Reference
Altricola flavicollis[a]	West Pakistan	10
A. roylei	West Pakistan	10
Apodemus agrarius	Siberia	11, 12
	Korea	9
A. sylvaticus	Tadzikistan	11
Bandicota bengalensis	Thailand	40, 41
	Burma, India	42
B. indica	Thailand	26, 40
	Taiwan	33
Callosciurus notatus	Malaya	8a, 43, 81a
Cricetulus triton	Siberia	11, 12
Crocidura lasiura	Siberia	11, 12
Hydromys humei	India	20
Hyperacrius fertilis	West Pakistan	10
H. wynnei	West Pakistan	10
Isodon macrorus	Queensland, Australia	44
I. torosus	Queensland, Australia	32
Melogale personata	Vietnam	45
Melomys cervinipes	Queensland, Australia	44
M. littorales	Queensland, Australia	32
M. lutillus	Queensland, Australia	44
Meriones tamariscinus	Tadzikistan	11
Microtis fortis	Siberia	11
	Korea	9
M. minutus	Korea	9
M. montebelli	Japan	3
Millardia metada	West Pakistan	10
Mus bactrianus	China	8
M. booduga	India	47
M. musculus	China	8
	West Pakistan	10
	Tadzikistan	11
	Taiwan	46
M. platyhrix	India	47
Nesioka indica	Tadzikistan	11
	West Pakistan	10
Rattus annandalei	Malaya	43
R. argentiventor	Malaya	43, 81a
R. assimilis	Queensland, Australia	32
R. blandfordi	India	47
R. bowersi	Malaya	43
R. conatus	Queensland, Australia	32
R. concolor	New Guinea	35
R. coxinga	Taiwan	33
R. edwardsi	Malaya	15, 43
R. everetti	Philippines	47
R. exulans	Philippines	47, 81a
R. flavipectus	Burma, India	48, 49
R. losea	China	8
R. leucopus	Queensland, Australia	44
R. mindanensis	Philippines	47
R. moi	Vietnam	45
R. mulleri	Malaya	15, 43
R. norvegicus[b]	Siberia	11

Table 2 (continued)
VERTEBRATES REPORTED INFECTED WITH *RICKETTSIA TSUTSUGAMUSHI*

Species	Locality	Reference
	Queensland, Australia	32
	Taiwan	46
	Japan	37
	China	8
R. panglima	Philippines	47
R. rajah	Malaya	43
R. rattoides	West Pakistan	10
R. rattus	West Pakistan	10
	China	8
	Taiwan	46, 51
	Indonesia	34
	India	20, 47, 52
	Burma	42
	Vietnam	45
	Thailand	40, 41
	Malaya	23, 43
R. sabanus	Malaya	23, 43
	Vietnam	45
R. surifer	Malaya	43
R. tiomanicus (=*R. jalorensis*)	Malaya	43, 81a
R. turkestanicus	Tadzikistan	11
R. whiteheadi	Malaya	43
Suncus murinus	West Pakistan	10
	India	47
Tatera indica	West Pakistan	10
Tupaia belangeri	Burma, India	20, 27, 49, 53
T. glis	Malaya	8a, 43, 81a
	Vietnam	45
Uromys caudimaculatus	Queensland, Australia	44
Cattle[c]	Eastern U.S.S.R.	53a

[a]The authors of the species are omitted since they are not given in most of the references cited.
[b]The *R. norvegicus caraco* referred to by Kulagin et al.[11] is considered to be *Rattus rattus caraco* according to Schwarz and Schwarz.[50] Traub and Wisseman[2] are of the opinion that the other *R. norvegicus* referred to in the literature are also probably forms of wild *Rattus rattus*.
[c]The authors[53a] consider cattle to be important "reservoir" hosts in the Far Eastern provinces of U.S.S.R. because of serological evidence of infection and the demonstration that *R. tsutsugamushi* survive for some time in them after inoculation. They also were able to induce three species of "*Trombicula*" to feed on cattle. The validity of this work is not known since it has not been confirmed.

from rodents, chiggers, and humans in West Pakistan,[80] however, showed that most were identical serologically to the Karp serotype, and the others were referrable to either the Gilliam or Kato serotypes. Analysis of 114 isolates from febrile patients in central (rural) Malaya by direct immunofluorescence tests revealed 29 antigenic types, with Karp and 3 Thai serotypes being the most common.[81,81a] The complexity of natural populations of *R. tsutsugamushi* may be even greater than the available data indicate. The prevalence and distribution of strains of low virulence for mice, the animal usually used for isolations, are poorly known since such strains are rarely recovered.[11,87–89]

Unlike the tickborne rickettsiae, *R. tsutsugamushi* serotypes do not appear to be geographically isolated. It is not unusual to find considerable variation among strains in even very small areas,[7,71,72,90] but Kitaoka et al.[17] found some correlation between serotype and species of chigger. In many of these studies, however, the time factor is not taken into account. As Traub and Wisseman[2] point out, habitats are not static, but are continually changing in contemporary as well as in geological time. This is particularly true of the subclimax formations where *R. tsutsugamushi* is commonly found. The scrub habitat is especially dynamic, continually and rapidly changing from the time of its creation often as a result of forest clearing, until the climax vegetation (forest) is reestablished or a new climax formation (e.g., lalang) becomes established. Successive stages of revegetation are accompanied by changes in the vertebrate and invertebrate fauna. In particular, the species of *Rattus* and trombiculid mites change as the vegetation types undergo progressive alteration. This could readily explain many instances of reported *R. tsutsugamushi* strain variation over time in certain situations.

Only a single species of *R. tsutsugamushi* is recognized, but it may be only one of a number of related species, most of which have not yet been discovered. An organism reported from Hokkaido, the northernmost island of Japan, was believed to differ enough from known strains to constitute a new species, *R. tamiyai,*[91,92] but later studies failed to show that it differed significantly from other strains of *R. tsutsugamushi* isolated from various sources throughout Japan.[70]

The agent Baker[93] isolated from Canadian voles seemed to be related to *R. tsutsugamushi,* but subsequent work has failed to substantiate it.[94–96] There is renewed interest in this and other rickettsia-like agents recovered from a variety of sources (see Volume II, Chapter 11). *Rickettsia sennetsu,* a rickettsia-like agent isolated from patients with infectious mononucleosis-like disease,[97] is morphologically similar to *R. tsutsugamushi,* but antigenically distinct.[98] It resembles chlamydiae more than it does rickettsiae, but it may be related to *Neorickettsia helminthoeca* (see Volume II, Chapter 9).

B. Arthropod Hosts

Rickettsia tsutsugamushi is a symbiote of one group of actinochitinose acarines, the Trombiculidae. The trombiculid mites are world-wide in distribution, but *R. tsutsugamushi* is restricted to parts of Asia, the Indonesian Archipelago, New Guinea, and northern Australia, where it is reported from only a few species in the genus *Leptotrombidium* Nagao et al., and to a lesser extent from *Ascoschongastia* Ewing, *Gahrliepia* Oudemans, *Eutrombicula* Ewing, and *Neotrombicula* Hirst.

1. Reported Isolations from Ticks

The report of an isolation of *R. tsutsugamushi* from *Ixodes* species ticks in Fukien Province, China[99] is difficult to evaluate. The most logical explanation is that the rickettsiae were in the blood meal ingested by the ticks from a rickettsemic host. There is some question that the agent is *R. tsutsugamushi* since the authors report that *Ixodes* ticks fed on infected mice transmitted it to rabbits, and fresh ticks fed on the infected rabbits transmitted it to rats, hamsters, and human beings.

A report on alleged scrub typhus rickettsiae in Sumatran ticks has already been referred to in Volume I, Chapter 5. In this case, the disease agents probably were tick typhus rickettsiae. *Rickettsia tsutsugamushi* may persist for some weeks in the hemolymph of experimentally inoculated ticks, but there is little evidence for multiplication, and the organism is not found outside the hemolymph and hemocytes.[100] An early report of transmission by ticks of the genus *Amblyomma*[101] can be discounted on technical grounds. A translation of a paper by Plotnikova and Fetisova[85] refers to isolation of strains from ticks, but this is almost certainly incorrect. The word for tick and mite is the same in Russian and the translator chose the wrong term.

2. Trombiculidae

The results of work by numerous investigators over many years is in remarkable agreement on two important points. The distribution of *R. tsutsugamushi* coincides with only two groups of animals: trombiculid mites of the genus *Leptotrombidium*, particularly *L.* (*Leptotrombidium*), and wild forms of the rodent genus *Rattus*, particularly the subgenus *Rattus*, both of which evolved in South and Southeast Asia. The origin and dispersion of the family Muridae and the genus *Rattus* in particular were outlined in Volume I, Chapter 4 and will be briefly discussed again in a later section of the present chapter. It is probably no accident that the center of distribution of the Muridae and the Trombiculidae is the tropical region of Asia and the Indonesian Island chain. Audy[102] lists 251 species of mammal chiggers in the Oriental and Australasian Region and estimates that there are nearly 400; Japan alone has about 60 species.[103] The genus *Leptotrombidium* contains some 50 to 60 species, with three fourths of them in South and East Asia.[104]

a. Origin and Disease Association

The origin of the Trombiculidae is not entirely clear, but they may have evolved from a common stem with trombidiid mites, some of whose larvae may have begun parasitizing insects, then cold-blooded vertebrates, and finally mammals and birds, as Audy[105] suggests. Or, as Ewing[106] believes, insect parasitism represents a later reversion among some groups, and parasitism among the larvae of the Trombidioidea first arose with parasitism of vertebrates. In any case, only the larvae are parasitic, feeding on tissue juices of a single host until engorged, then dropping to the ground litter where they complete their life cycle as free-living predators. It is not known for certain when or where the trombiculids diverged from their free-living ancestors. The family is represented on all the major land masses of the world with the possible exception of Antarctica, but in terms of number of genera and species, the tropical rain forest of Southeast Asia is currently a major center of distribution.

Much of our knowledge about *R. tsutsugamushi* is based on studies stimulated by and strongly oriented towards the disease it causes in man. Larvae of the major vectors of chiggerborne typhus (*L. akamushi, L. deliense, L. fletcheri, L. arenicola, L. pallidum,* and *L. scutellare*), readily attack man and appear to be efficient transmitters of the disease. We know something about the host-parasite relationships (rickettsia-mite and mite-vertebrate) of these trombiculids, but comparatively little about such relationships in the hundreds of other trombiculid species that do not attack man. However, it may be significant that *R. tsutsugamushi* has been found only where certain *Leptotrombidium, Ascoschongastia,* and *Gahrliepia* occur. Chigger mites that bite man outside the range of these three genera (more specifically *Leptotrombidium*) have never been found to transmit chiggerborne typhus or any disease remotely resembling it.

b. Leptotrombidium

The genus *Leptotrombidium* is widely distributed throughout South and Southeast Asia, and occurs in the Palearctic, Nearctic, and Ethiopian, as well as in portions of the Australasian Regions. It is a dominant genus on ground-living mammals and some birds over the entire chiggerborne rickettsiosis endemic region.[107] The species most commonly associated with transmission of chiggerborne rickettsiosis are in the subgenus *Leptotrombidium: L.* (*L.*) *deliense* (Walach), *L.* (*L.*) *akamushi* Brumpt, *L.* (*L.*) *fletcheri* (Womersley and Heaslip), *L.* (*L.*) *arenicola* Traub, *L.* (*L.*) *pallidum* (Nagayo et al.), *L.* (*L.*) *scutellare* (Nagayo et al.), and *L.* (*L.*) *pavlovskyi* (Schluger). Other species are undoubtedly involved in the maintenance of natural cycles of *R. tsutsugamushi* (Table 1). Readers interested in the taxonomy, biology, and ecology of Asian and Southeast

Asian trombiculids should consult Audy,[102,108] Wharton and Fuller,[109] Womersley,[16] Womersley and Audy,[110] Harrison,[111] Harrison and Audy,[31] Suyemoto and Toshioka,[112] Sasa,[113,114] Sasa and Jameson,[115] Jameson and Toshioka,[116] Vercamman-Grandjean,[117] Traub and Nadchatram,[118,119] Traub et al.,[120] Neal and Barnett,[121] Nadchatram,[122] Traub and Wisseman,[123] Shirasaka and Sasa,[124] Audy and Lavoipierre.[125]

Leptotrombidium deliense—*Leptotrombidium deliense* is a very adaptable species, and is found in a variety of habitats throughout its vast range from southern China to Queensland in Northern Australia. It is especially adapted to secondary forest-scrub habitat, but may occur in abundance in grassy areas associated with recently cleared forests, along stream banks, and in disturbed forest.[123,126–128] It has been implicated as a vector or maintaining host of *R. tsutsugamushi* throughout its range. The first strains isolated from mites in Malaya were from pools of *L. deliense* and pools containing a mixture of *L. deliense* and *L. fletcheri* (reported as *L. akamushi*.)[129] Two of the mixed pools were composed of mites collected from quail (*Excalfactoria c. chinensis*). This is the first recorded instance of isolation of the agent from mites collected from birds. In West Pakistan, Traub et al.[10] found *L. deliense* transmitting chiggerborne typhus in the lowland district of Sialkot. It was not prevalent in the high Himalayan valleys of West Pakistan. Varma[130,131] has reported it to be widespread in the Himalayas of West Bengal and Sikkim up to 3840 m elevation, but Traub and Wisseman[2] question the correctness of the identification of these collections and consider them to have been other species of *Leptotrombidium* which, at the time, were undescribed.

Leptotrobidium akamushi—The classical vector of *R. tsutsugamushi* in Japan is *L. akamushi,* which is found only in restricted habitats along the banks of rivers and streams.[3] All reference to *L. akamushi* elsewhere in Asia and Australasia apparently are in error and probably refer to *L. fletcheri,* misidentified *L. deliense,* or possibly undescribed species.[2] Miyajima and Okumura[132] initially demonstrated that monkeys fed on by larvae of *L. akamushi* reared in the laboratory from wild parents developed a febrile disease indistinguishable from what was known as tsutsugamushi disease. Thus, the authors showed (on clinical grounds) not only that *L. akamushi* is naturally infected, but also that it can transmit the infection by bite and that the organism is transovarially transmitted in this mite.

Leptotrombidium fletcheri—*Leptotrombidium fletcheri* is closely related to *L. deliense* and is nearly as widely distributed. It ranges from Southeast Asia to New Guinea but has not been reported from Australia and is uncommon or absent in India. Both *L. fletcheri* and *L. deliense* may occur in the same habitat and even together on the same host, but *L. fletcheri* is primarily found in habitats where grasses and herbs predominate.[127–129,133]

Leptotrombidium arenicola—*Leptotrombidium arenicola* is restricted to the margins of sandy beaches and in nearby scrub vegetation in Malaya and the islands off the west coast.[134] It can transmit *R. tsutsugamushi* in the laboratory[135] and is probably a major vector throughout its range.[22,123]

Leptotrombidium pallidum—*Leptotrombidium pallidum* (=*Trombicula pallida*) is a major vector in Japan during the winter and early spring[3] and also has been implicated as a vector in Korea[9] and in the Primorye Region of the U.S.S.R.[38,136,137] In Korea, strains of *R. tsutsugamushi* were isolated from laboratory-reared larvae of adult females collected in the field. Isolations made from laboratory mice on which the larvae had been fed demonstrated the ability of *L. pallidum* to transmit the rickettsia to a vertebrate host and to transmit it transovarially to succeeding generations.

Leptotrombidium scutellare—*Leptotrombium scutellare* (=*Trombicula scutellaris*) transmits *R. tsutsugamushi* in Japan during the fall and winter.[2,37] Attempts to isolate the rickettsia from this species in its mountain habitat in Malaya were not successful.[2]